Behavioural Factors Influencing Orientation and Navigation in Homing Pigeons (*Columba livia*)

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Zusammenfassung

Brieftauben welche über hunderte von Kilometern von ihrem Schlag entfernt aufgelassen werden kehren oft noch am gleichen Tag zurück. Diese bemerkenswerte Fähigkeit beruht nicht auf einer Suche nach dem Zufallsprinzip, sondern auf echter Navigation. Diese basiert auf einer Positionsbestimmung in Bezug zum Heimschlag, und einer daraus abgeleiteten Kompassrichtung zum Schlag.

Wie die Tauben ihre Position auf einer grossräumigen Koordinatensystems wahrnehmen oder bestimmen ist immer noch ungeklärt. Die Faktoren welche die gewählte Heimrichtung beeinflussen können waren aber Objekt vieler Studien. Dabei erscheinen drei Faktoren von Bedeutung. Uebereinstimmend wird akzeptiert, dass die Tauben zur Ermittlung der Heimflugrichtung den Sonnenstand benutzen, belegt durch zahlreiche Experimente mit Verstellung der inneren Uhr. Weiterhin erscheint die Stärke des geomagnetischen Feldes als zusätzliches Hilfsmittel zur Bestimmung der Heimrichtung bei bedeckten Himmel, weil experimentelle Störungen der Magnetfeldwahrnehmung zu einem etwas beeinträchtigten Heimfinde-Erfolg führten. Schliesslich erscheint das olfaktorische System von Bedeutung, weil Tauben mit durchtrenntem Geruchsnerv oder langdauernder Anosmie nach Auflässen in unbekanntem Gebiet häufig nicht mehr nach Hause finden.

Zahlreiche Studien und Beobachtungen haben jedoch gezeigt, dass die obigen Orientierungsmechanismen durch zusätzliche Faktoren beeinflusst werden. So benutzen Brieftauben topographische Merkpunkte zur Optimierung oder Korrektur der Heimflugrichtung. Insbesondere ziehen es alleinfliegende Tauben vor, entlang topographisch orientierter Leitstrukturen anstatt in der direkten Heimrichtung zu fliegen.

Die Richtungsbestimmung der Brieftaube sowie der Heimkehrerfolg wird zusätzlich durch Verhaltensfaktoren beeinflusst. Diese umfassen Sozialverhalten, frühere Erfahrungen sowie Unterschiede in der Heimkehrmotivation. Allerdings wurden diese Faktoren bislang kaum systematisch untersucht.

Das Ziel dieser Studien war somit das systematische Erfassen von richtungsbestimmenden Verhaltensfaktoren, das Ermitteln deren realen und relativen Einflüsse auf die Taubennavigation, sowie das Erfassen möglicher Interaktionen. Dazu wurden drei Studien mittels GPS Pfadaufzeichnung durchgeführt.

In der ersten Arbeit wurde die Heimkehrleistung von Tauben verglichen, die entweder allein oder in einer kleinen Gruppe flogen. Unsere Resultate zeigten dabei

einen positiven Effekt des Gruppenfluges: Kleingruppen flogen schneller als Alleinflieger, machten weniger Flugpausen, and zeigten effizientere Flugpfade. Darüberhinaus folgten Tauben im Gruppenflug weniger sichtbaren topographischen Leitstrukturen. Die impliziert dass der soziale Zusammenhalt den Konflikt zwischen der Wahl einer direkten Heimflugrichtung und einer Landmarkennavigation zugunsten der Richtungsnavigation beeinflusst.

In einer zweiten Arbeit wurde geprüft, inwieweit vorgängige Trainingserfahrung die Richtungswahl beinflusst. Dabei wurden zwei Gruppen von Tauben entlang entgegengesetzten Richtungen zum heimschlag vortrainiert, und dann über dem Meer von einer Position aus aufgelassen, von der aus die ideale Heimflugrichtung rechtwinklig zu den trainierten Richtungen zum Schlag verlief . Dabei zeigte ich ein starken Erfahrungseffekt, weil die Tauben über dem Meer in Richtung des Vortrainings wegzogen. Nach Erreichen der Küste korrigierten sie dann die Richtung um den Heimschlag zu erreichen.

Die dritte und letzte Auflassstudie setzte sich zum Ziel, Motivationsfaktoren zur Verbesserung der Richtungsfindung und des Heimkehrerfolges zu erfassen. Dabei stellte sich heraus, dass die Wartezeit bis zum Auflass einen wichtigen Faktor darstellt. Jene Tauben die am längsten in der Transportkiste warten mussten flogen schneller nach Hause und verzichteten auf langes Kreisen am Auflassort. Andererseits waren die Parameter für die Richtungsoptimierung während des restlichen Heimfluges unverändert. Dies könnte darauf beruhen, dass die Verbesserung der Flugzeit nur motivationsbedingt waren, und nicht auf einer Beinflussung der Orientierungsfähigkeit beruhte.

Zusammengefasst zeigte sich dass der Gebrauch von GPS Pfadaufzeichnungsgeräten es erlaubte, auch die Einflüsse von Sozialverhalten, Erfahrung und Motivation auf die Navigationsfähigkeit der Tauben zu erfassen. Damit eröffnen sich neue Perspektiven für Untersuchungen zum Heimfindevermögen und Navigationsmechanismen.

Summary

Homing pigeons released hundreds of kilometres from the home loft often return within the same day. Their remarkable performance is not due to random search but reflects true navigation based on the perception of their position relative to the loft and the ability to calculate a compass direction towards the loft.

While it is still poorly understood how pigeons can sense their position on a large-scale map, many studies have tried to understand factors used by homing pigeons for determining the home direction. Three main elements seem to provide important cues. First of all, there is a general agreement that pigeons use the position of the sun in establishing a compass at the release site as revealed by clock-shift experiments. Secondly, the geomagnetic field seems to represent an additional/alternative compass when the sun is not visible because disturbing the pigeons' magnetic perception apparently resulted in moderately impaired homing. Finally, olfaction appears to represent another important cue, because pigeons with sectioned olfactory nerve or with temporary or long-lasting anosmia are often unable to home when released from unfamiliar sites.

However, numerous experiments and observations revealed that other factors do have an influence on the orientation mechanisms described. Pigeons use landmarks to home faster and to correct wrong homing directions. In particular, when flying alone, they prefer following available longitudinal landmarks pointing home (such as roads) rather than using a direct compass course.

In addition, there are behavioural factors that could have an effect on the overall homing performance and directness of flights. These include sociality, previous experience and the motivation to home, but they have received little or no attention so far.

Therefore, the aim of this study was to systematically investigate these behavioural influences, to evaluate their real and relative importance in pigeons' navigation and the possible interactions between them. We performed three main experiments.

In the first one, the homing performance of individual homers was compared to the performance of small flocks. Our results showed a positive effect of flocking: flocks flew faster to the loft, without stops and with more efficient paths. Moreover, flock flying pigeons showed significantly less road-following, suggesting that companionship shifts the balance between landmark and compass navigation toward the latter. In the second

experiment, we tested the influence of experience by training two groups of pigeons from opposite directions with respect to the loft, and releasing them afterwards from a release site in a perpendicular direction, in the middle of the sea (20 km from the coast). The results evidenced a strong effect of experience as all birds had a heavy bias in flying over the sea according to the direction of previous training. Once over land they started to correct their compass course and to head home. In the third and final experiment, we tried to identify causes of variable homing motivation. One turned out to be the waiting time prior to release. Pigeons that waited the longest in their transport box before a release had faster flight and homing times and circled less around the release site. At the same time, measures of path efficiency remained unchanged, suggesting that the improvement was due to motivation only and that the general navigation capabilities were unmodified.

In conclusion, the use of GPS loggers allowed to assess the influence of behavioural factors such as sociality, experience and motivation on navigation capabilities and homing performances of pigeons and opened new perspectives in the study of homing and navigation.

Introduction

Homing pigeons (*Columba livia forma domestica*) have been bred by man starting from the wild pigeon, the rock dove (*Columba livia forma selvatica*). The selection, made during centuries, was according to their capacity and speed of homing after a passive displacement (Schmidt-Koenig 1980). Modern strains of homing pigeons are able to come back to their loft after displacements of several hundreds of kilometres, even from completely unknown territories. Their remarkable capacities do not reflect a random search of the home loft but are due to true navigation: pigeons are able to determine their position and to calculate the course to follow to reach their home loft (Schmidt-Koenig 1980). The ease of breeding and of experimental manipulations made homing pigeons the perfect candidate as model species for scientific studies on orientation and navigation, to match with studies using migratory species.

How do pigeons navigate? The historical background

Kramer (1957) described in his Map-and-Compass model avian homing as composed of two fundamentally different steps: one is establishing the position of the release place relative to the home loft (the map step), the other is determining the direction of flight (the compass step). Before the formulation of this theory, Kramer (1950; Kramer 1952) had already detected that birds can use the sun to determine compass directions. As the sun position changes over time, its usability for spatial orientation requires the involvement of a clock. Hoffmann (1954) showed that the bird's internal clock can be shifted by a change of the light-dark cycle and that this temporal shift results in a corresponding angular shift of the bird's compass. Initial bearings of pigeons that had been living for several days in a light-dark cycle shifted by 6 h either forward or backward, deviated from those of non-shifted controls by roughly 90° counterclockwise or clockwise, respectively (Schmidt-Koenig 1958; Schmidt-Koenig 1961)(Fig.1). These experiments, which clearly demonstrated the use of the sun as a compass, are still considered a milestone in the study of orientation and navigation.

However, pigeons, as other navigating birds, remain able to home and orient also under overcast skies, when the sun is not visible. This raised the question about the existence of a different compass system. An alternative compass mechanism based on the geomagnetic field was first described for European robins, *Erithacus rubecula*, a

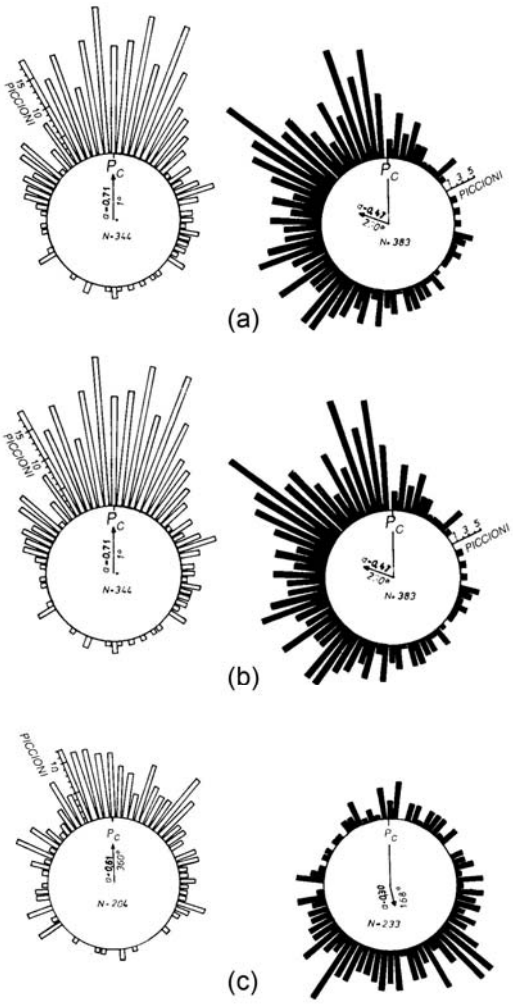

Figure 1. Initial bearings of control pigeons (white) compared with clock-shifted pigeons (black) (a) 6 hours clockwise, (b) 6 hours counterclockwise and (c) 12 hours. Each radius gives the relative number of pigeons disappeared in that direction (piccioni). Initial bearings are given respect to control pigeons (Pc). (Figure from Schmidt-Koenig 1980)

migrant species (Wiltschko 1968). However, since a sense organ for magnetoreception so far has not clearly been identified, the classical way of examining the importance of a given sensory input by interfering with the related sense organ or its neural connections cannot be used. Thus, experiments are restricted to artificial alterations of magnetic fields to which the birds are exposed and to correlations of the bird's behaviour with naturally occurring magnetic irregularities. Experiments on homing pigeons were mainly conducted applying strong bar magnets on their back or on their head (Keeton 1971; Ioalè 2000), or equipping them with Helmoltz coils (Visalberghi and Alleva 1979). Taken together, such experiments "seem to provide evidence that under certain conditions magnet can confuse homing pigeons" (Keeton 1971). This cautious sentence implies that confusion does not occur consistently or under clearly defined conditions. Under sunny conditions, the pigeons appeared only exceptionally confused by magnets or coils, albeit some moderate increase in angular dispersion of initial bearings was frequently observed. Under overcast skies, in contrast, disorientation caused by magnets or coils seems to be the rule, but cases of no effect were also observed (for a complete review see Wallraff 2005). Thus far, the only report of massive impairment by attached magnets has been provided by Harada (2002) who applied the magnets close to the lagena, an otholithic structure found only in birds and fishes. However, even in this case, it is not clear whether the magnetic field bothered the birds or caused malfunction of the vestibular system.

In the model proposed by Kramer the map step remained entirely uninvestigated. The term “map” is often poorly defined. It does not necessarily imply that a bird has a representation of a coordinate system or any other wide-ranging spatial configuration in its brain. It merely means that there are environmental factors whose two-dimensional spatial order involves information on distant positions relative to a bird’s home site. How a bird makes use of such information is not automatically implied (Wallraff 2004). The only demonstrations of a true mental map are largely unpublished studies by the group of Lipp (Lipp 1996). They reported that pigeons released from an unknown site were oriented either to the home or to a feeding loft, depending on their motivational status.

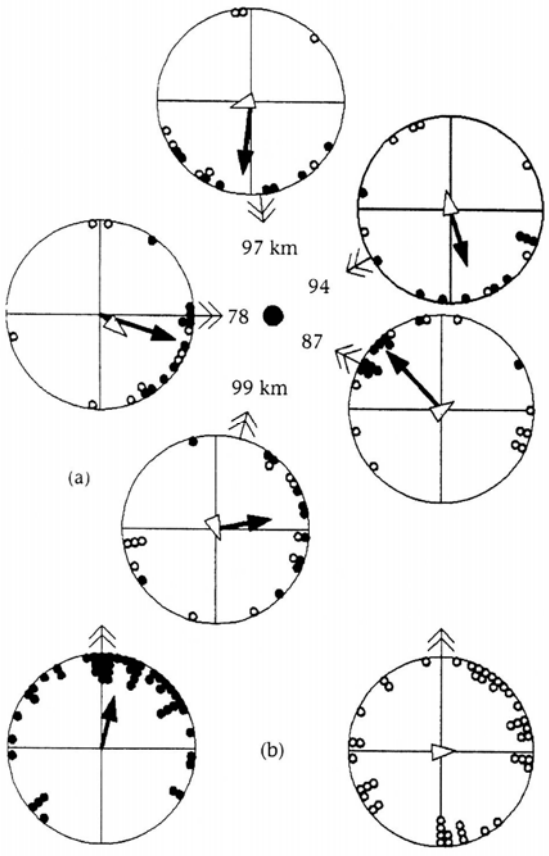

Figure 2. (a) Vanishing bearings at the five sites 78-99 km distant from a home loft near Florence, Italy. (b) The same data pooled with homeward direction pointing upwards. All birds with one bisected olfactory nerve and one occluded nostril. *Filled symbols*: both treatments on the same side (controls); *open symbols*: treatments on different sides (experimental birds). (Modified from Papi et al. 1980)

In 1971, Papi and collaborators demonstrated for the first time a fundamental role of the olfactory system for homing using the method of nerve sectioning (Papi et al. 1971). Although the simple and brief procedure of nerve cutting does not cause any side effects in everyday behaviour such as walking, flying, perching, breeding, etc., it is an invasive method. Therefore, in subsequent experiments the problem of possible side effects was taken into account by combining unilateral nerve cutting with unilateral plugging of a nostril. The two treatments were applied contralaterally in the experimental birds and ipsilaterally in the controls. Thus, all birds experienced the same interference with the nervous system and with breathing (Papi et al. 1972; Papi et al. 1980). Figure 2 shows results obtained with this method: only the experimental birds failed to orient their initial courses homeward whereas the control birds oriented homeward like untreated

pigeons in other cases. The conclusions were that the map that enables the pigeon to return from unknown places is of olfactory nature and is acquired by associating the odours carried by the wind with its direction of origin (Papi et al. 1972). Since then, numerous studies in which the pigeons' sense of smell or airborne molecules or winds were altered continued to support the fact that olfactory signals are indispensable for homing from unfamiliar areas (for reviews see Papi 2001; Wallraff 2004; Wallraff 2005).

Taken together, these homing mechanisms are referred to a long-distance navigation believed to be based on geophysical or air-borne gradients.

The role of the visual landscape

Wild rock pigeons are colony breeders and depart for daily flights to feeding sites up to 30 km and return from there (Schmidt-Koenig 1980). Given the excellent visual system of pigeons, it is likely that homing pigeons learn, during their free flights (leading them up to 15 km away from the loft), visual features of the landscape such as mountains, hills, forests, lakes, rivers, villages and towns. Also, it seems very likely that pigeons make active use of such features for orientational purposes once they are familiarized with the spatial relationships between them (Wallraff 2005).

The use of landscape features and landmarks for navigation has been subject of numerous debates. Birds with frosted lenses were capable of straight flights towards the loft, even if the use of landmarks seemed crucial at the very final stage of finding the loft itself (Schmidt-Koenig and Walcott 1978). On the other hand, pigeons were able to find the loft in very dark nights, suggesting that they can make use of non-visual cues even relatively close to the loft (Lipp, 1983).

However, whether pigeons can make use of large-scale topographic features in order to determine their position at a remote unfamiliar release site is not clear yet. The use of landmarks over familiar terrains was first supported by indirect evidence from the results of clock-shift experiments in the familiar area in which the deflection of vanishing bearings was reduced than expected. Since the reduced deflection was only present at familiar site and not at unfamiliar ones, this indicated that birds probably use landmarks for piloting (Wallraff et al. 1999). Another important evidence for the use of landmarks was that birds made anosmic cannot orient from unfamiliar sites but are able to do so from familiar ones (Benvenuti et al. 1973; Hartwick et al. 1977). Finally, a reduced or absent deflection was often shown when clock-shifted birds were made anosmic and thus could not use olfactory cues to navigate with the sun compass (Bingman and Ioalè

1989; Wallraff et al. 1994; Gagliardo et al. 1999). These experiments have generally been interpreted that the reduced deflection was caused by the influence of familiar visual landmarks independent of the map and compass system.

The introduction of GPS loggers

The development of GPS technology for homing pigeons, developed jointly by the group of Lipp and the Swiss Federal Institute of Technology (Steiner et al. 2000), allowed to follow the homing pigeons during their entire flight home, reconstructing the flight path with high temporal (1 fix per second) and spatial (spatial error < 4 m in 95% of fixes) resolution and allowing to overlap these paths to topographic maps or to high resolution satellite maps. This new possibility allowed seeing that, when repeatedly released from the same location, pigeons are able to recapitulate previous flight paths, developing stereotyped routes (Biro et al. 2004). Moreover, when available, pigeons use and follow main longitudinal landmarks pointing home, such as roads and railways (Lipp et al. 2004) (Fig. 3).

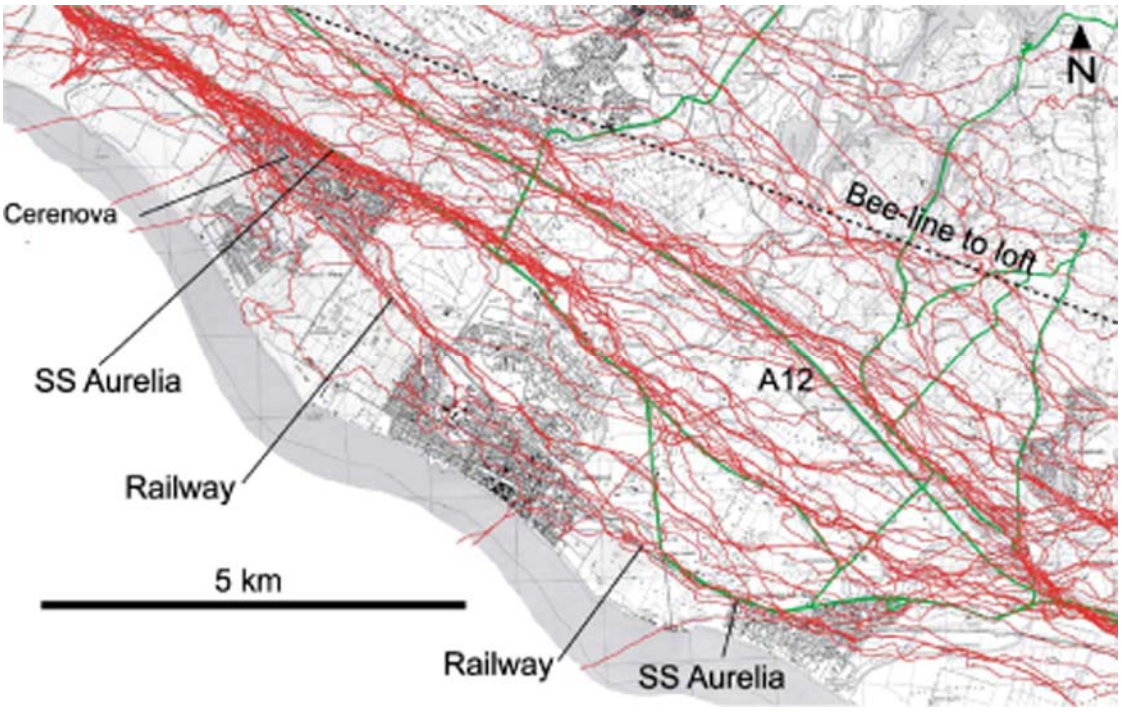

Figure 3. GPS tracks of pigeons homing along motorways and railways. The beeline to the loft shows the compass direction from the main release site in the northwest. Note an apparent conflict of strategies; pigeons appear to be attracted by both longitudinal objects in parallel to the beeline and by the proper compass direction. This conflict appears in two features: pigeons shift preferentially to the left of the highways when these turn away from the compass direction, and there are frequent break-offs of tracks subsequently aligning with the motorway A12 or toward the beeline. Green lines show GPS tracks obtained by car. (Figure from Lipp et al. 2004**)**

The use of GPS loggers confirmed also the fact that landmarks are mainly important in the so-called familiar home range as experienced by the birds during their free flight or after repeated releases from familiar sites. Experiments with “prisoners” pigeons, reared and kept confined into an aviary until the experimental release, showed that a limited view of the home area impairs the pigeons’ ability to relocate the loft at their first homing flight, suggesting that the final step of the homing process is mediated by visual recognition of familiar landmarks in the home area (Gagliardo et al. 2007). Most recent studies have shown that the pigeon’s hippocampus appears to play a role in assembling a visually based mental map of the familiar home range (Gagliardo et al. 2009).

The emerging hypothesis is that flying pigeons, released from a familiar site, can choose between following a rather precise compass direction (using their long-distance homing strategy), or alternatively follow landmarks providing a suboptimal but predictable way home.

Other influences on homing performances

Despite the numerous studies on orientation mechanisms and on the use or not use of landmarks as potential feature influencing orientation and homing, until now, little or no scientific attention was paid on other possible factors playing a role in the final homing performance. A scheme depicting the main orientation mechanisms, influencing factors, and the relations among them, is shown in Fig. 4. Out of these complex interactions, this thesis shall focus mainly on three factors: the effects of flocking, the effects of training and previous experience, and the effects of homing motivation at the release site.

The effects of flock flying and of previous experience have already been slightly investigated in the past but, in both cases, without the employment of GPS loggers giving the possibility to record the entire flight. Most conclusions were, therefore, based on three parameters only: vanishing bearings during the first 2-3 km, vanishing times and homing times. Such parameters measure the behaviour of the bird at departure at the release site and at arrival at the loft but there is no information depicted during the rest of the homing journey.

Concerning the effects of flock flying, mathematical models on animal navigating in groups state that “the pooling of information from many inaccurate compasses yields a single more accurate composite-compass because individual orientation errors are mutually corrected by group cohesion” (Simons 2004). However, such predicted improvement has been observed only in some studies (Hamilton 1967; Tamm 1980),

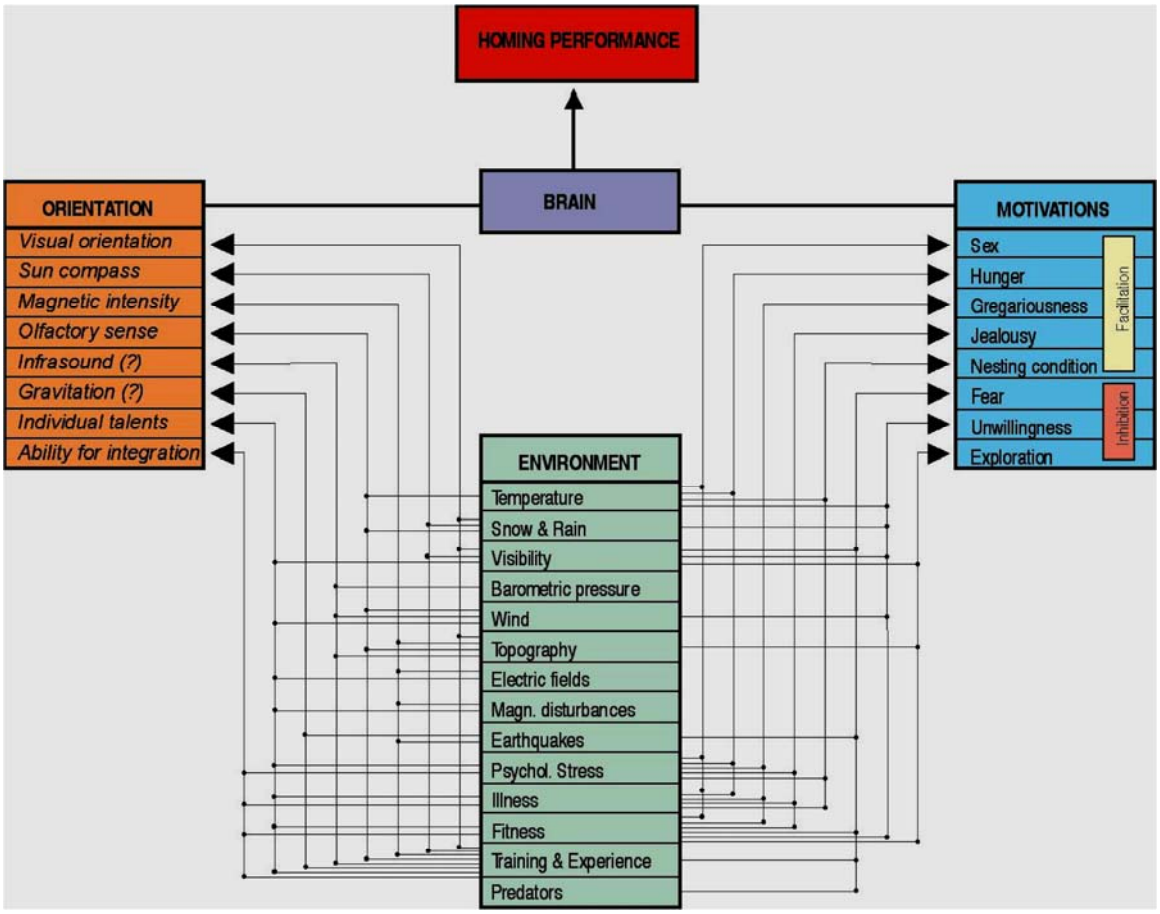

Figure 4. Basic mechanisms of orientation and possible factors of influence in the homing behaviour of pigeons. (Modified from Lipp 1996)

while in others no difference between performances of individuals and groups was detected (Keeton 1970; Benvenuti and Baldaccini 1985). On this issue, the gap in the recording of the homing flight could be the main cause for the controversial results obtained.

Previous experience was found to improve homing performance (Wallraff 1967). When pigeons are repeatedly released from the same site, they improve their direction of initial bearings and their vanishing and homing speed. However, this is only observed during the first three-to-six releases; afterwards the homing performance reaches an asymptote that does not change with further releases (Graue 1965; Wallraff 2005). Despite the numerous studies on the effects of increased familiarity with an area and the relative increase in homing performance, no experiments investigated the effects on orientation of repeated releases from the same direction once the birds are released from new locations.

Finally, that motivational aspects have an influence on homing performance has not received scientific attention so far. Pigeons' breeders are well aware of the importance of motivation as this is a main factor in successful racing. They have successfully developed differential strategies (i.e. food reward, manipulations of the brood or the clutch, etc.) to improve the homing motivation of displaced pigeons and the homing performance during competitions. However, despite the empirical knowledge of the fanciers, amassed during the past hundred years, scientists have almost completely ignored this issue. Only very recently, motivational factors are being considered at least in the discussion of results of homing pigeon studies (Biro et al. 2002; Schiffner and Wiltschko 2009).

Aims of the study

The aim of this study is to investigate these three behavioural factors having an influence on compass orientation, understanding their relative importance in the homing process and identifying possible relationships and interactions between them. Moreover, the use of GPS loggers shall allow a detailed reconstruction of flight paths, filling the gap of the majority of previous studies between vanishing at the release site and arrival at the loft. The first study is planned to understand whether flocking has a positive effect on orientation as predicted by models and how homing behaviour is influenced by it, comparing the performances of pigeons repeatedly released as individuals and in a flock. The second experiment is aimed to find the effects of previous directional training when pigeons are released from a new release site, in the middle of the sea, in a perpendicular direction to that of the training. Finally, the third study intends to find out whether the waiting duration at the release site has an effect on homing motivation and homing performance.

Study area and facilities

The study took place in the facilities of the University of Zurich at Testa di Lepre, Italy, 25 km NW of Rome (12.28° N; 41.93° E). There, in a traditional farm setting, local homing pigeons were housed in 3 identical mobile lofts equipped with aviaries (formerly Swiss

Army) and cared for by an experienced breeder. Pigeons of both sexes and with different flying experience were living in the same loft. All birds were habitually allowed to fly freely outside the lofts and they underwent regular training, which entailed frequent handling.

This particular location has been chosen for two main reasons. The first is the reduced number of raptors in this area. Birds of prey are considered almost a pest by pigeons breeders in Switzerland because of the high number of pigeons predated during releases and even at the loft. Important losses during releases pose a problem in the interpretation of results, but also an economic one when many GPS-carrying pigeons are lost. The second reason is the possibility to perform releases from the sea, which is only 10 km far from the loft. Over the sea there are no landmarks or other visual cues that pigeons can use to orient towards home; in such conditions pigeons are obliged to rely predominantly on their compass sense. This is a particular and useful situation to understand possible factors influencing compass orientation.

Chapter 1

Flock flying improves pigeons' homing: GPS track analysis of individual flyers versus small groups.

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Flock flying improves pigeons' homing: GPS track analysis of individual flyers versus small groups.

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ABSTRACT

The effects of aggregation in navigating animals have generated growing interest in field and theoretical studies. The few studies on the effects of group flying on the performance of homing pigeons, *Columba livia*, have led to controversial conclusions, chiefly because of the lack of appropriate technology to follow pigeons during their entire homeward flight. Therefore, we used GPS data loggers on six highly trained pigeons from a familiar release site first by releasing them six times individually, then six times as a group from the same site, and, finally, again six times individually. Flight data showed that the homing performance of the birds flying as a flock was significantly better than that of the birds released individually. When flying in a flock, pigeons showed no resting episodes, shorter homing times, higher speed, and almost no circling around the start zone in comparison to individual flights. Moreover, flock-flying pigeons took a nearly direct, 'beeline' route to the loft, whereas individually flying birds preferred to follow roads and other longitudinal landmarks leading towards the loft, even when it caused a detour. Our results show that group cohesion facilitates a shift towards more efficient homing strategies: individuals prefer navigating by familiar landmarks, while flocks show a compass orientation.

INTRODUCTION

Many animals spontaneously aggregate when foraging or when travelling. Aggregation is commonly recognized to provide benefits for group members, for instance through predation avoidance or improved foraging efficiency (Krause & Ruxton 2002). Recently, there has been an increasing interest in the potential navigational advantages for animals moving in groups (Simons 2004; Conradt & Roper 2005; Couzin et al. 2005; Hancock et al. 2006; Codling et al. 2007).

According to the 'many-wrongs principle' (Bergman & Donner 1964; Hamilton 1967; Wallraff 1978; Simons

2004), group cohesion allows a more accurate navigation because individual errors are mutually corrected through information pooling. This advantage of group navigation has found further support from theoretical models showing that even experienced and informed individuals have a larger navigational error than the combined error of several inexperienced group members (Conradt& Roper 2003).

Homing pigeons, Columba livia, provide an optimal model for navigation research owing to their well-developed orientation capabilities and the ease of their experimental manipulation (Schmidt-Koenig 1980). Experimental studies have demonstrated the existence of different orientation mechanisms (reviewed in Walcott 2005). While there are conflicting theories with respect to orientation mechanisms used by pigeons, the most widely accepted notion is still Kramer's (1957) map-and-compass model. It holds that displaced birds first determine their position (the map step) and then follow a homeward course (the compass step). Ideally, this is the beeline from release site to the loft. Calculation of this compass direction includes the position of the sun (if visible) and, presumably, magnetic cues.

Pigeons that are repeatedly released from the same location generally improve their homing performance, reaching an asymptote after three to six releases (Graue 1965; Wallraff 2005). On the other hand, GPS tracking studies have shown that repeated releases from a familiar location result in stereotyped routes during homing (Biro et al. 2004), often along longitudinal landmarks such as roads and railways (Lipp et al. 2004).

The role of group flying in homing performance has been investigated in a limited number of studies, and these have led to conflicting conclusions. Some of these studies suggested that orientation in a flock is more accurate than that of individual birds (Hamilton 1967), with less-scattered vanishing bearings and shorter homing times (Tamm 1980). In contrast, other experiments failed to show any improvement in

navigational accuracy of pigeons released in flocks (Keeton 1970; Benvenuti & Baldaccini 1985). Some of these contradictions may reflect the fact that these early studies assessed directional information only at the release site, namely vanishing bearings, and homing speed as the only performance variable.

The development of small GPS data loggers now permits precise reconstruction of the homeward journey of pigeons (Steiner et al. 2000; Von Hünerbein et al. 2000; Biro et al. 2002; Lipp et al. 2004), and thus a reassessment of the problem.

In the present study, we compared homing performances of the same pigeons successively released individually, in a flock, and again individually, always from the same site. All pigeons were highly trained from that release site to avoid increasing familiarity confounds of release repetitions. None the less, if flock navigation is superior, one would expect an increase in homing performance in pigeons released in flocks, and a subsequent performance drop upon reverting to the individual-release schedule, even from a familiar release site.

METHODS

Study Area and Facilities

Homing pigeons used for this study were kept in the facilities of the University of Zurich at Testa di Lepre, Italy, 25 km northwest of Rome (12.28_N, 41.93_E). There, in a traditional farm setting, local homing pigeons were housed in three identical mobile lofts equipped with aviaries (formerly Swiss Army) and cared for by an experienced breeder. Pigeons of both sexes and with different flying experience lived in the same loft. Food (a mixture of various cereals, peas, corn and sunflower seeds sold commercially for racing pigeons), grit and water were provided ad libitum. All birds were habitually allowed to fly freely outside the lofts and they underwent regular training, which entailed frequent handling. During training, the birds were transported to various locations in all directions up to 50 km from the loft and released in small flocks or individually. The study was licensed by the Ministero della Salute Roma.

Subjects and General Procedure

All the experimental releases took place between November 2005 and April 2006 under sunny conditions, with no or light wind, from the release site Santa Severa (11.98°N, 42.03°E), 27 km northwest of the home loft.

In this experiment we used six adult 2-year-old pigeons (four males and two females) which had been released from Santa Severa up to 20 times before the present experiment took place and, thus, were in the asymptotic phase of their homing performance (see also Graue 1965; Wallraff 2005).

Between experimental homing releases, the six birds always wore PVC dummy weights (22 g, 4e5% of body weight), affixed on their backs with Velcro strips to habituate them to the load. To mount dummies or loggers, we trimmed the dorsal feathers between the wings in a small area of 1.5 _ 3 cm. A strip of rough plastic Velcro was glued on the trimmed feathers with nontoxic contact glue and we made sure that the strip and the attached dummy did not interfere with the pigeons' movements and flight. The soft part of the Velcro was glued on to the dummies and GPS loggers. We separated the load from the dorsal Velcro by inserting a flat tool between the two strips, to avoid ripping off any feathers. Pigeons naturally lost the glued Velcro with the moult. For experiments, the dummies were replaced with GPS loggers of the same weight (NewBehavior AG, Zurich, Switzerland) just before the release, and placed on the birds again after we retrieved the GPS at the loft. The logger took one positional fix every second, and then stored the data. Further technical information can be found in Biro et al. (2002) and Lipp et al. (2004).

The birds first underwent six individual releases (S1) from a starting crate to establish baseline performance. Releases took place at intervals of 3 days. Subsequently, the same birds were released from the same crate as a flock (F), again at intervals of 3 days for a total of six releases. This served to assess possible improvements caused by flock navigation. Finally, they underwent six further individual releases (S2) to determine to what extent they would maintain the performance level of flock navigation.

Data Analysis

The raw data were downloaded from the logger to a computer and analysed first for possible artefacts and irregularities of recording (program Wintrack, freeware, D.P. Wolfer at www.dpwolfer.ch/wintrack; Steiner et al. 2000; Wolfer et al. 2001). The program then extracted the following variables: homing speed (average speed recorded by the GPS logger during flight, excluding measures of speed of less than 5 km/h), flight altitude, number and duration of rests (rests were defined as episodes longer than 5 s with GPS speed less than 5 km/h), total flying time, average distance to the beeline between the release site and the loft, and distance flown (km) flown along the main

roads and the coast (episodes of road or coast following were defined as flying parallel to or at an angle of <10° to the road/coastline at a distance of 200 m or less for at least 500 m).

We also calculated the straightness index, D/L, for each track, in which D is the beeline distance from the starting point to the goal, and L is the path actually followed by the animal (Batschelet 1981; Benhamou 2004). This is a scale-independent measure and, given the high recording frequency of one positional fix/s, a reliable estimator of the efficiency of the orientation process already used by other authors (i.e. Biro et al. 2004).

These parameters were analysed with parametric and nonparametric procedures. In a first step, simple Pearson correlations were used to check whether the first series of six individual releases showed any improvement over asymptotic performance during consecutive releases (x = order of releases per condition, y = averaged score of the six birds). This procedure was also applied to the other conditions to discover any effects of repeated releases.

To analyse differences between the three conditions, we averaged the values from the S1, F and S2 conditions, because the number of repeated factors in a one-way ANOVA design (18 here) should not exceed the sample size (N = 6). These averaged values were then used for a nonparametric one-way ANOVA with three repeated factors (S1, F, S2; Friedman test for related samples, two-tailed), followed post hoc by pairwise nonparametric comparisons (Wilcoxon test for related samples). Predictions were that the group flight condition would reveal better performance, and that comparisons between S1 and S2 should show either no differences or improvement only. Thus, one-tailed significance levels were applied. For simplifying data presentation, the Friedman ANOVA values are omitted in figures and text. We analysed individual variation in the six pigeons graphically by plotting three key variables (flight speed, straightness index and road following) for each of the 18 releases.

We used the software package Statview 5.01 (SAS Institute, Cary, NC, U.S.A.). To plot GPS tracks we used MapInfo (Map Info Corp., Troy, NY, U.S.A.).

RESULTS

Overall, we conducted 107 releases of the 108 planned (six pigeons released six times in each of the three series of releases) with the GPS data loggers and obtained complete and technically valid tracks from all but two tracks in the S1 series (p613,

p830). For the last individual release in S2, one pigeon (p811) was excluded because it had sustained injuries during the fifth release.

Figure 1 summarizes the main results in the form of GPS tracks. The tracks of singly released birds, before and after group flights, were generally well oriented, but showed considerable topographical scattering to the left and right of the beeline (a direct line between release site and loft). Prior to the group flights, this scatter was mainly towards the right side of the beeline in a region rich in longitudinal landmarks pointing home, such as roads and railways. In fact, as indicated by overlapping flight paths, the pigeons showed road following mostly along the motorway A12.

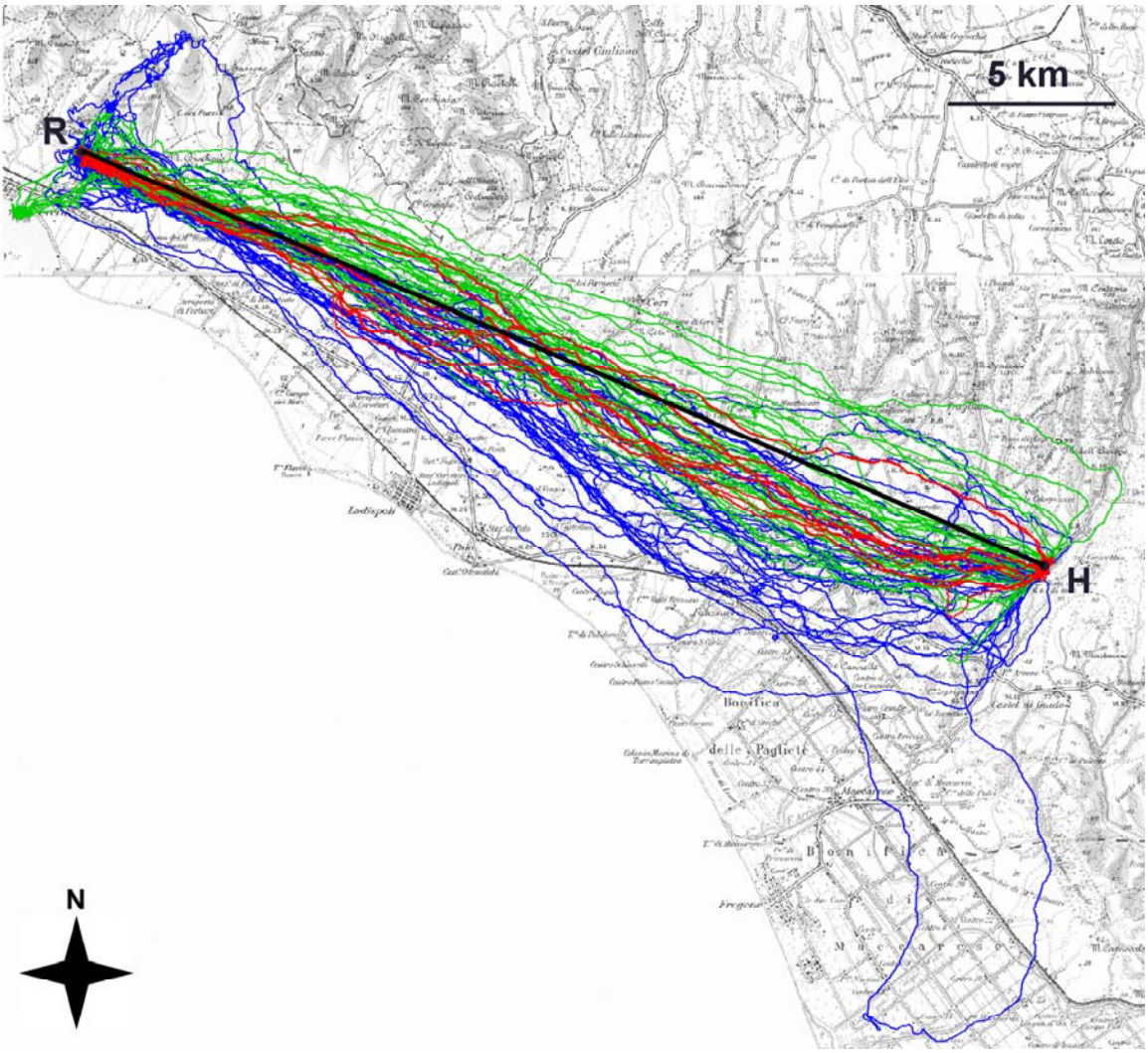

Figure 1 GPS tracks of homing pigeons between the release site (R) and the home loft (H). Blue tracks: 36 individual flights of six experienced pigeons released six times, condition S1. Red tracks: six group releases of the same six pigeons as a flock (apparent as one track per release because pigeons did not split from the flock), condition F. Green tracks: 35 individual flights performed after the group releases, condition S2.

When the same pigeons were released in groups of six, they flew much closer to the beeline, but always followed somewhat different trajectories. In three of the six releases, the pigeons flew close together, from the release point to the loft; in two releases the birds flew together but they split 1-3 km before the loft, following individual routes, partially along a local road. During the first group release, the flock divided after about 10 km into individually flying birds; the particular path of splitting suggests a raptor attack. However, they kept a relatively parallel course, not moving more than 1 km from each other, and they again formed a cohesive flock during subsequent flight, the last pigeon rejoining the group about 7 km after the splitting. Thus, the splitting of the terminal trajectories, and during the first group release, caused minor quantitative within-group variation in the analysis of flight parameters.

In the individual releases subsequent to the group flights, S2, the flight trajectories appeared again much more scattered. A number of flights appeared to have shifted to the north into a region that does not contain structural cues leading homewards. Some overlapping of tracks (implying development of new route preferences) was noted in these regions, too, albeit less than in the S1 condition.

The comparison across the six successive releases of S1 for each individual pigeon failed to detect any systematic trend in repeated flights, indicating that the pigeons had already reached asymptotic (yet not invariant) homing performance from this familiar site. Three of the birds (p601, p811, p823) showed high but not temporally ordered variability in flight speed, straightness index and road following, while the others (p613, p830, p848) performed relatively constantly (Fig. 2).

The overall comparison of flock-versus-individual releases revealed significant differences in a number of variables. When pigeons were group released they invariably flew to the loft without any periods of resting. In contrast, when released individually some of the pigeons took a rest on the way home (Fig. 3a). Moreover, the actual flight speed recorded by the GPS loggers showed that flocks flew faster than did most of their members during individual S1 releases, with the exception of one release when pigeons p601 and p811 flew faster than the flocks (see also Fig. 2). During flock flights, speed increased significantly over releases (Pearson correlation: $r_4 = 0.82$, $P < 0.05$; x = order of releases, y = average speed of birds per release). Individual birds then maintained this average group flight speed during the S2 releases (Wilcoxon signed-ranks test, one-tailed: T = -2.2, N = 6, P = 0.014 for SI versus F and SI versus S2; Fig. 3b), possibly indicating a physical training effect.

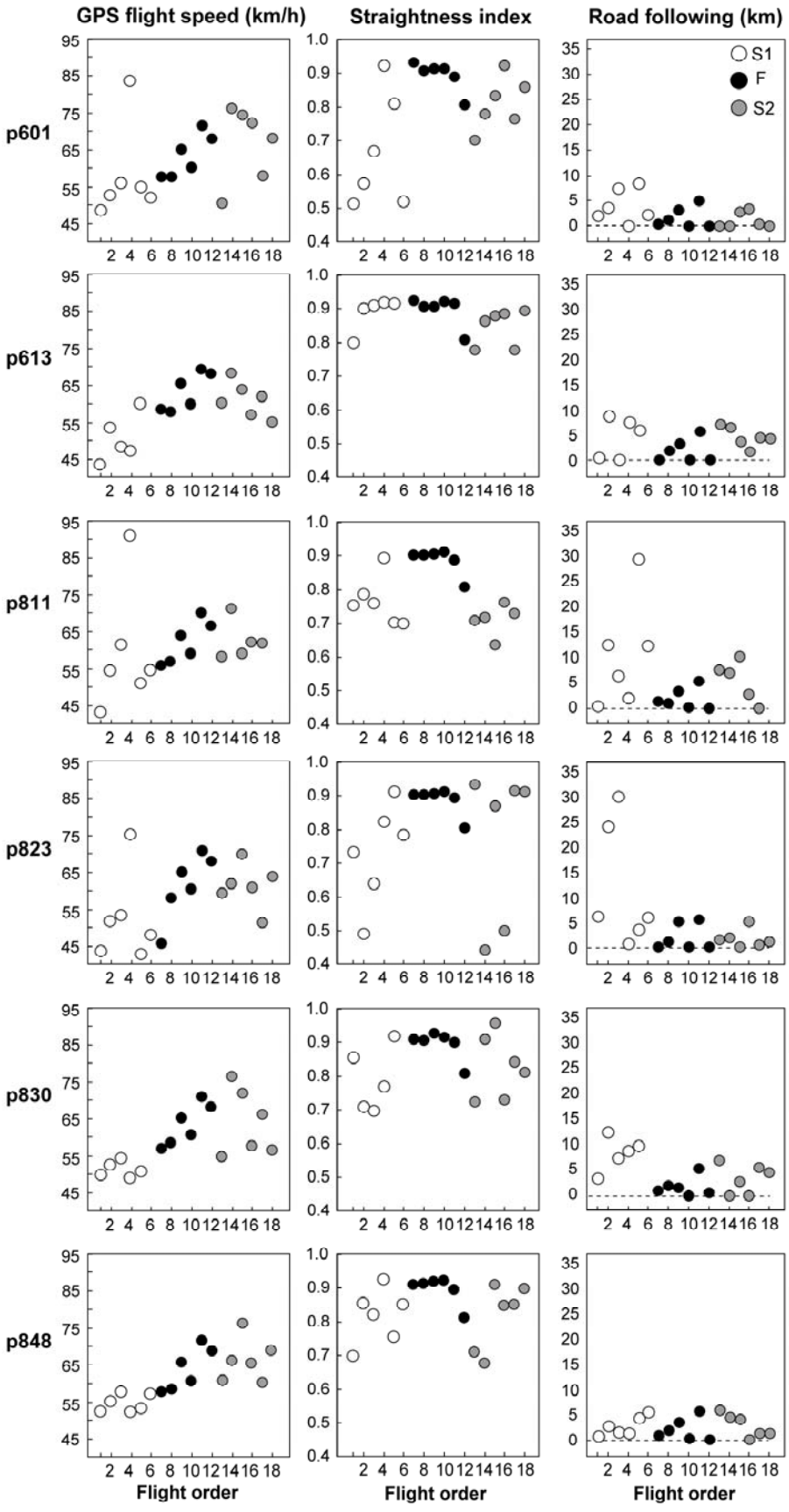
GPS flight speed (km/h)
Straightness index
Road following (km)
S1
F
S2
p601
p613
p811
p823
p830
p848
Flight order
Flight order
Flight order

Figure 2 Individual scores for homing speed, straightness index and road following across different releases plotted for the six pigeons (p601, p613, p811, p823, p830, p848). The corresponding but averaged values per condition and related statistics are shown in Figs. 3b, 4a and b. All Y values show the same scale for comparison. White dots: first series of individual releases (condition S1); black dots: flock releases (condition F); grey dots: second series of individual releases, performed after the flock releases (condition S2). (Pigeons p613 and p830 have five S1 releases because of a corrupted track recorded by GPS; p811 has five S2 releases because it sustained injuries.)

Measures of path geometry revealed a more efficient navigation for group flights; the path to leave the start zone (defined as the distance flown before leaving a circle of 1 km radius about the release point) was significantly shorter when pigeons flew as a flock than in the two series of individual releases (T = -1.99, N = 6, P = 0.023 for S1 versus F and F versus S2). There was no significant difference between the two series of individual releases (Fig. 3c). Similarly, the straightness index was significantly higher in flocks, indicating a more linear way home (T = -2.2, N = 6, P = 0.014 for S1 versus F and F versus S2), than in both series of individual releases, with no statistical difference between the latter (Fig. 4a). The average distance of the track from the beeline between release site and loft was shorter when pigeons were flying as a flock than in the first series of individual releases (T = -2.2, N = 6, P = 0.014). Again, S2 pigeons showed an average increase of the distance to the beeline compared to the F1 condition but this was not significant (T = -1.57, N = 6, P = 0.058).

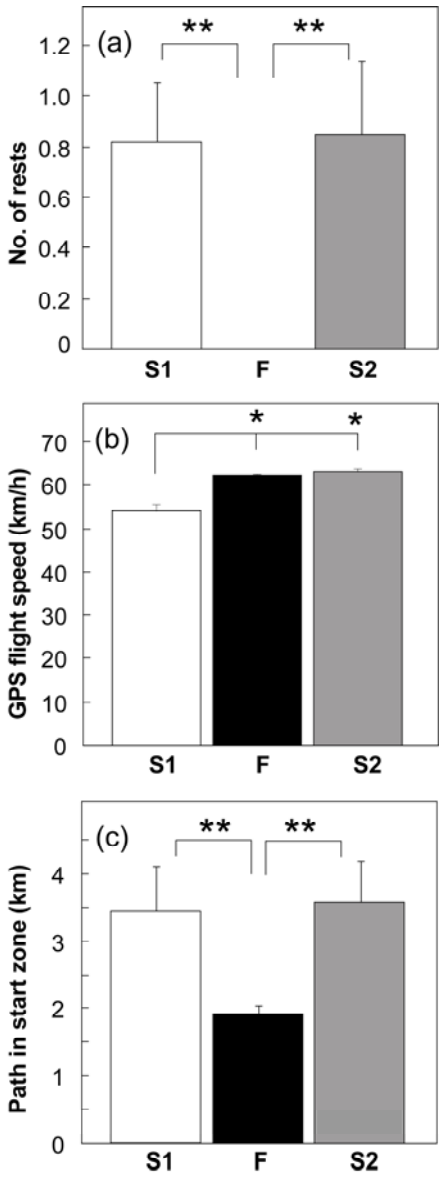

Figure 3 (a) Average number of rests during flight. (b) Average homing speed recorded by the GPS logger. (c) Path to leave start zone (defined as the distance flown before leaving a circle of 1 km radius around the release point). Bars indicate means + SEM. **P < 0.025. S1: individual flights; F: group flights; S2: individual flights after group releases.

To find reasons for the prolonged flight paths of singly flying birds, we also measured the total cumulative length of flight tracks along longitudinal landmarks, such as roads and coastline (known to be followed by pigeons released from this place, Lipp et al. 2004). Individually flying pigeons in S1 flew along the main roads (particularly the motorway) significantly more than flock-flying pigeons (T = -2.2, N = 6, P = 0.014). In the S2 condition, road following showed a nonsignificant tendency to increase compared to the F condition (T = -1.57, N = 6, P = 0.058; Fig. 4b). An analysis of Pearson correlations, however, showed a significant reduction of road following over consecutive releases (r_4 = -0.87, P < 0.05; x = order of S2 releases, y = average road-following scores per release). No differences were observed in flight altitude.

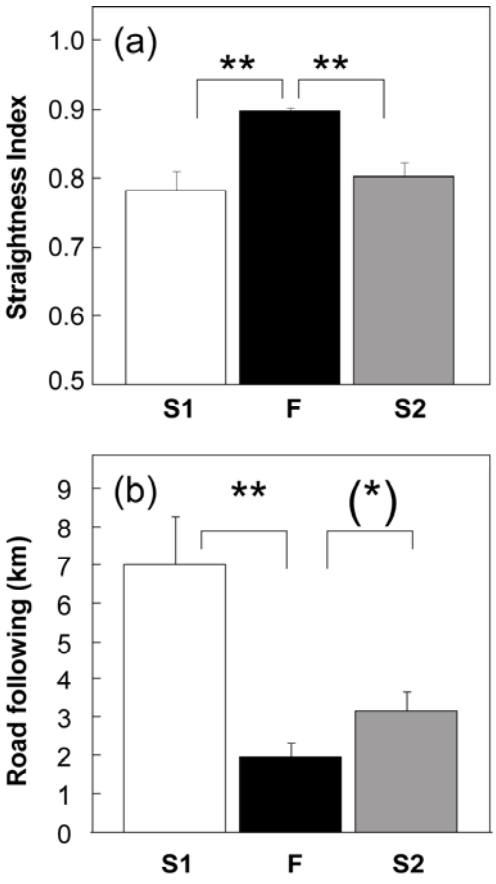

Figure 4 (a) Straightness index. (b) Road-following scores. Bars indicate means + SEM. **P < 0.025. S1: individual flights; F: group flights; S2: individual flights after group releases.

A graphical inspection of individual variation in three key variables (flight speed, straightness index and road following; Fig. 2) largely confirmed the results of the ANOVA using averaged data, but revealed some interesting aspects. For example, two pigeons (p601 and p811) showed, during the fourth S1 release, high flight speed and a flock-like straightness index. During the following release, however, they were much slower and had a high road-following score.

Between-release variation of measures in the flock condition showed a much more homogeneous performance than for both individual-release conditions. However, a clearly lower straightness index was observed for the last of the group releases, indicating a suboptimal group trajectory on that day, although homing speed and road following were not affected. A detailed analysis of GPS tracks revealed that the flock, while approximately following the beeline, performed a series of loops and turns over the first 3 km from the release site, as was often observed in singly released pigeons.

The analysis of individual transitions from flock releases to individual releases showed that flight speed and straightness index dropped most distinctly during the first or second release after flock conditions, during which four pigeons also increased their road-following score. Thereafter, four of the six pigeons (p601, p613, p823, p848) regained a straightness index that was comparable or only slightly inferior to the flock condition. While this temporary impairment resulted in significant (nonparametric) group differences for the averaged values between the F and the S2 condition, it also indicates that the pigeons did not lose their ability for well-directed homing.

DISCUSSION

Our results demonstrate superior homing performance of pigeons released in small flocks compared to pigeons released individually, even when tested in releases from a highly familiar location. In comparison to individual flights, pigeons in a flock left the release site faster, flew generally faster, made no stops, and showed improved directionality during their homeward flight. This confirms the predictions of the many-wrongs principle and other models of group navigation predicting cancelling of individual navigational errors (Bergman & Donner 1964; Simons 2004; Codling et al. 2007).

In this study, the homing performance of pigeons was a compound of initial flight behaviour at the release site, actual flight speed, number of rests and navigational accuracy during homing. It is unlikely, however, that all these parameters can be classified only as mutually cancelling navigational errors. Prolonged circling around the release site may be taken as an indicator of directional uncertainty, but since the release site was thoroughly familiar, it is more likely to reflect the tendency to wait for a companion bird. Similarly, stops during flight may be caused by orientation problems, by lack of flight motivation or, again, by waiting for a companion. The changes in these two variables suggest, at least in part, motivational problems associated with the individual flight condition, particularly as they were observed after successive fast and efficient flock homing. Thus, flying in flocks appears, somehow, to increase homing motivation. This conclusion is supported by the observation that reverting from flock to individual flight condition reduced homing performance during the first release of the S2 condition, while pigeons attained levels comparable to flock flight afterwards, mostly regarding homing speed.

On the other hand, the improvement in directionality observed in flock-flying pigeons, and the lower variability of all measured variables, is in agreement with superior flock

navigation predicted by group navigation models (Bergman & Donner 1964; Simons 2004; Codling et al. 2007). However, in such models directional errors are assumed to be random. In our case, the directional error was a systematic bias introduced by previous development of individual stereotyped routes, typically observed after repeated releases from a familiar location (Biro et al. 2004; Lipp et al. 2004). The reasons underlying development of stereotyped routes are still unclear. These directional biases cannot be actual navigational errors (the birds return reliably), but may be a suboptimal homing strategy. Nevertheless, flock flying significantly reduced such individual directional biases. Based on these findings, one can probably expect larger corrections by group flights in releases from unfamiliar sites, where the probability of true navigation errors is higher.

Occasionally, individually flying pigeons were able to show almost perfect homing in terms of directionality and speed. This indicates that individually flying pigeons, released from a familiar site, can choose between following a rather precise compass direction and following landmarks providing a suboptimal but predictable way home. In the majority of cases, pigeons flying alone seemed to prefer such route following, whereas this strategy was only occasionally shown by flocks. Thus, flying in flocks appears to shift the balance between homing strategies in favour of compass navigation which is always used by pigeons from unfamiliar sites.

Homing pigeons have an innate tendency to group when flying because of their evolution and breeding history (Schmidt-Koenig 1980), and group cohesion is actively kept. GPS tracks show that groups rarely split, and, if they do, subgroups may separate up to 1 km before joining each other, as observed during the first group release. At least in small flocks, group cohesion prevents individual flock members landing and resting and also drives pigeons to adopt flight speeds they would not maintain while flying alone. Future research should investigate whether there are changes in some measurable physiological parameter, such as physical effort or stress, among pigeons released individually or in flocks.

Why flock-flying pigeons abandon acquired route strategies in favour of (superior) compass orientation is unknown. One possible explanation is that flock-flying pigeons must pay visual attention to their companions to maintain flock cohesion, thus cancelling the attraction of landmarks, and possibly also the influence of other distracting visual cues. In consequence, the flock maintains the compass direction to the loft better than individually flying pigeons. This idea needs to be tested, but preliminary data from EEG

recordings in flock- versus individually flying pigeons shows less attentional EEG responses of flock-flying birds when passing familiar landmarks (Vyssotski et al. 2007).

A possible alternative explanation of superior homing performance of flocks is the presence of a leader bird with better navigational abilities, leading the companions home. Since the precision of the GPS used did not allow us to test this hypothesis directly, we checked for every release the rank order of the pigeons according to their performance. In the case of a typical leader dictating the speed and direction of the flock, the leader bird should have consistent performance in individual and group flights. However, we failed to identify a pigeon with constant superior performance. This observation corresponds to previous results showing increased performance in all pigeons (Benvenuti & Baldaccini 1985; Biro et al. 2006).

In conclusion, flying in small flocks has an important positive effect on homing performance, in terms of navigational accuracy, speed and motivation, even in releases from highly familiar release sites. GPS tracking shows that pigeons can shift dynamically between coexisting strategies: individually flying pigeons rely more on topographical features for homing, keeping habitual home routes, whereas flocks tend to adopt a compass-based navigation.

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Chapter 2

The influence of experience in orientation: GPS tracking of homing pigeons released over the sea after directional training.

Journal of Experimental Biology 212: 178-183

The influence of experience in orientation: GPS tracking of homing pigeons released over the sea after directional training

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ABSTRACT

Flight experience is one of the factors that influences initial orientation of displaced homing pigeons (*Columba livia*). Prior studies showed a systematic dependence of initial orientation on previously flown direction. Using GPS data loggers, this study sought to examine the effect of previous directional training of 40 homing pigeons when they were released over the sea, in the absence of proximal landmarks, in a direction almost perpendicular to that of previous training flights. Our results demonstrated that previous directional training evoked a systematic and predicted deviation from the beeline over the sea that appeared as a compromise between the direction of training and the direction to the loft. Pigeons were able to efficiently correct their flight direction only once over land, where they flew significantly slower and less directly than over the sea.

INTRODUCTION

Individual experience, gained from previous homing flights, is one of the various factors that influences the initial orientation of displaced homing pigeons (*Columba livia*) (Wallraff, 1967). Pigeons benefit from repeated releases from the same site, considerably increasing their homing performance, especially during the first three-to-six releases (Graue, 1965; Wallraff, 2005). Moreover, GPS tracking studies showed that increased familiarity with a release site entails stereotyped routes during homing (Biro et al., 2004), often along longitudinal landmarks roughly pointing home, such as roads and railways (Lipp et al., 2004).

Despite numerous studies on the effects of increased familiarity with an area and the relative increase in homing performance, few experiments have investigated the effects on orientation of repeated releases in the same direction, once the birds are released from new locations. Wallraff (Wallraff, 1974) found that there is a systematic dependence in the vanishing bearings on the direction of previous experience: released pigeons tend to fly in the same direction previously flown, albeit with high individual variation. He hypothesized that the amount and availability of external information for orientation influence the magnitude of the deviation: the more such information is available, the less pigeons rely on previously flown direction. Moreover, he also hypothesized that the navigational process itself could be affected. In other words, the effect of experience might not only result in a directional preference but it could also influence the manner in which the parameters composing the birds' 'map' are processed. According to the 'Map-and- Compass' model (Kramer, 1957), displaced birds first determine their position relative to the home loft based on locally available cues (the map step) and with the aid of olfactory stimuli (Papi, 1991; Wallraff, 2005), they then calculate a homeward course (the compass step) with the aid of the position of the sun (Kramer, 1953) and, presumably, magnetic cues (Wiltschko and Wiltschko, 2003).

The importance of landmarks for orientation is a controversial subject and it remains unclear to which degree they can contribute in the determination and learning of a homeward direction (for a review, see Walcott, 2005); however, numerous studies demonstrated that over familiar territories pigeons follow longitudinal landmarks when available and prefer to fly over areas rich in landmarks (Biro et al., 2004; Lau et al., 2006; Lipp et al., 2004). Moreover, landmarks seem to stabilize the flight direction, even over unknown territories (Lipp et al., 2004).

In this study, we investigated the effect of directional training on orientation of homing pigeons in a context of absence of landmarks. We first trained two groups of pigeons from one of two land release sites located in opposite directions from the loft. We then released these birds individually from a boat at sea, from a point located at an almost perpendicular direction from training flights. Previous experiments have shown that pigeons forced to cross water surfaces chose the shortest way to the coast but also that the general flight direction is not affected once the birds have recognized the home bearing (Wagner, 1972). We selected a release site at sea equidistant from land in all directions over a 100 degree angle bisected by the beeline to the loft. We expected that, if experience gained with directional training entails a biased compass orientation, pigeons will follow the training direction and will reach the coast either to the left or right of the beeline, respectively. Once the coast has been reached, the continuation of flights according to the training direction would indicate a predominant role of directional learning on orientation. On the contrary, a correction to the right homewards direction would indicate the capacity to determine the correct home bearing, gained either during the flight to the coast or from additional terrestrial cues.

MATERIALS AND METHODS

The homing pigeons (*Columba livia* Gmelin 1789) used for this study were all young (approximately six months old) with no previous homing experience. They were located in the facilities of the University of Zurich at Testa di Lepre, Italy, 25km NW of Rome (12.28 deg. N; 41.93 deg. E). Pigeons of both sexes and with different flying experience inhabited the same loft. Food (a mixture of various cereals, peas, corn and sunflower seeds sold commercially for racing pigeons), grit and water were provided *ad libitum*. All birds were habitually allowed to fly freely outside the loft but none of the experimental pigeons were transported to any other location before the actual experiment.

The entire experiment was carried out between March and July 2006. Forty pigeons in total, both males and females, were equipped with dummies to habituate them to fly and live with the load (22 g, 4–5% of total body mass). The dummy weight was a small piece of PVC, of the same size and mass of the GPS data-logger, which was affixed on their backs with Velcro® strips, following a procedure already described by Biro et al. and Dell'Ariccia et al. (Biro et al., 2002; Dell'Ariccia et al., 2008).

The experiment took place in two stages, due to the availability of GPS loggers. A group of 20 pigeons that had no previous release experience were trained as follows:

two flock releases from two sites, 5 km and 10 km distant from the loft, respectively, in the same direction as that of the final release site – Castel Romano (12.44 deg. N; 41.71 deg. E), which was 27 km SE from the loft. From here, pigeons were then released twice as a flock, four times in pairs and six times individually. At the end of this training, every pigeon was released 14 times, always from the same direction with respect to the home loft. During the last two individual releases, we equipped birds with GPS loggers to track their home route. After training, all 20 pigeons were released individually from the sea. The sea release site (11.99 deg. N; 41.77 deg. E) was 30 km SW from the loft and 20km from the coast at a point between two small promontories, which were at the same distance from the release site – always at 20km; moreover, the beeline between the sea release site and the loft was perpendicular to the coastline. The angle included between the direction of previous training and the beeline between the sea release site and the loft was 80 deg.

The procedure was then repeated with a new sample of inexperienced pigeons but now from a release site opposite to the first release site. These 20 pigeons had the same age, sex and experience as the first group. They were accustomed to wearing dummies and had received exactly the same training procedure as that of the first group but were released from sites NW from the loft until being released from the final site – Santa Severa (11.98 deg. N; 42.03 deg. E), which was 27 km NW from the loft. Again, after directional training, pigeons were released individually from the sea, from exactly the same site 30 km from the loft. In this case, the angle included between the direction of previous training and the beeline between the sea release site and the loft was 60 deg.

In the last two individual releases from land and in the releases from sea, pigeons were equipped with GPS loggers (www.technosmart.eu): the dummies were replaced by GPS dataloggers just before the release and replaced again on the birds after retrieving the GPS at the loft. The loggers took one positional fix every second and then stored the data. Further technical information can be found in Lipp et al. (Lipp et al., 2004).

All releases took place in sunny conditions for both groups. Birds were released for training with no or light wind; both sea releases took place in days with no wind at all. The total absence of wind was also subsequently verified using the daily ocean wind data by satellite recording that are freely available on the website: http://podaac.jpl.nasa.gov/DATA_PRODUCT/OVW/index.html (Product 109).

During training releases, we lost three pigeons from the NW release site whereas during the sea release, two pigeons got lost – one previously trained from the NW and one from the SE. During the sea release, one GPS logger failed to record and one pigeon returned without his GPS logger; in both cases, the pigeons were previously trained from the NW. Therefore, we finally analyzed 19 tracks of pigeons previously trained from the SE and 14 tracks from the NW.

The raw data were downloaded from the GPS loggers to a computer and analyzed first for possible artefacts and irregularities of recording (program WINTRACK. Freeware D. P. Wolfer at www.dpwolfer.ch/wintrack) (Steiner et al., 2000; Wolfer et al., 2001). The program then extracted the following variables: homing speed (HS: average speed recorded by GPS logger during flight, excluding measures of speed of less than 5kmh–1), flight altitude (ALT), total number (TNrst) and total duration (TTrst) of rests (rests were defined as episodes longer than 5 s with GPS or ground speed less than 5kmh–1), total flying time (TTfly), direction of deviation (DEVlin) and average distance (ADlin) to the beeline between the release site and the loft, and vanishing error (VE: the difference, in degrees, between the vanishing bearing 1 km from the release point and the loft direction).

We also calculated the straightness index (SI) for each track as D/L, in which D is the beeline distance from the starting point to the goal (or the difference between initial and final beeline distance to the loft in case of incomplete tracks) and L is the total path length flown (Benhamou, 2004). This is a scale-independent measure and, considering the high precision of path reconstruction at 1 fix s^{-1}, a reliable estimator of the efficiency of the orientation process.

The variables obtained by WINTRACK and the SI were subsequently statistically analyzed in two different ways: those with a normal distribution (HS, TTfly, DEVlin, ADlin, VE) and those normalized (ALT) were analyzed using the unpaired Student's *t*test whereas the other parameters that had a non-normal distribution (TNrst, TTrst, SI) were analyzed with the Mann–Whitney *U*-test to compare the effects of different directional training. Normality was tested using the Kolmogorov–Smirnov test.

To investigate possible changes in flight parameters during flight, we cut all tracks at the point where they cross the coastline then compared HS and SI in the path over the sea and over land within each individual flight. To calculate the SI of the segments, we applied the previous formula considering the point of intersection of each track with the coastline as the goal for the sea segments and as the starting point for the land

segments. In this way we obtained the real index values for every track segment. We used the paired *t*-test and the Wilcoxon test for HS and SI, respectively.

Analyses were performed using the software package STATISTICA 7™ and with the aid of http://www.physics.csbsju.edu/stats/ (Kirkman, 1996). Plotting of GPS tracks was done with the aid of MapInfo™.

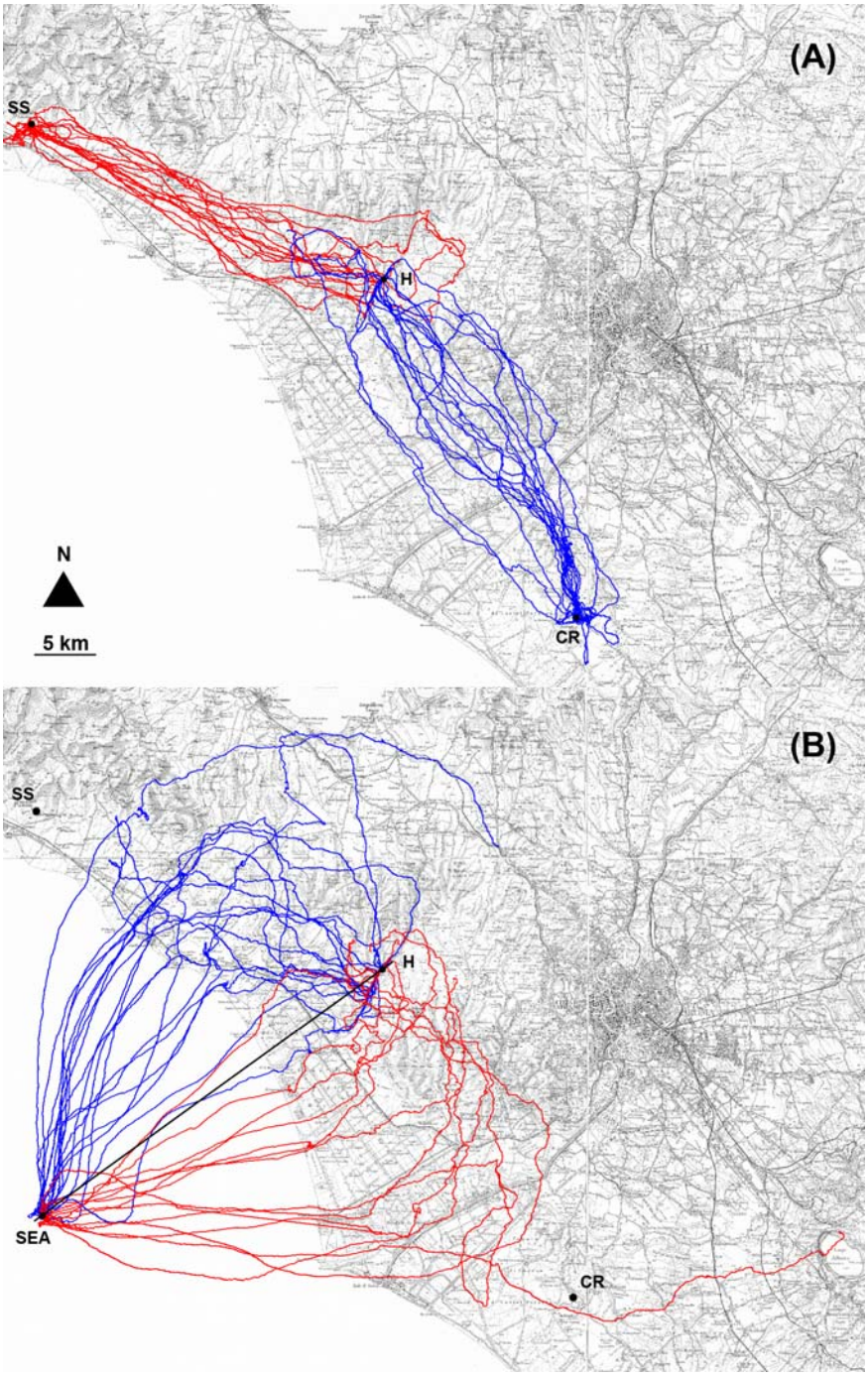

Fig. 1. GPS tracks of homing pigeons (A) during their last training release and (B) during the experimental sea release (SEA). Red tracks: pigeons trained from the release site SS, north-west of the home loft (H). Blue tracks: pigeons trained from the release site CR, south-east of the home loft.

RESULTS

We observed a remarkable difference in the orientation over the sea between homing pigeons from the two different training groups. Results are summarized in Fig. 1, which shows the well-oriented tracks of pigeons during their last training release (Fig. 1A) and the biased orientation in the sea release (Fig. 1B).

Considering sea tracks (Fig.1B), it appears evident that all pigeons were deeply influenced in their orientation by the direction of previous training: none of the pigeons flew in the opposite direction, and the beeline between the release site and the loft marked an almost complete separation between the groups. However, within both groups there was a certain degree of variability in the amount of deviation from the beeline to the loft.

Most of the pigeons (22 out of 33) crossed the coast over one of the small coastal towns present in this region (Fig. 2). Looking from the sea, towns appear as the most evident and proximate feature of the coastline. This suggests that pigeons could have directed their flight towards conspicuous (even if unknown) points of the coastline.

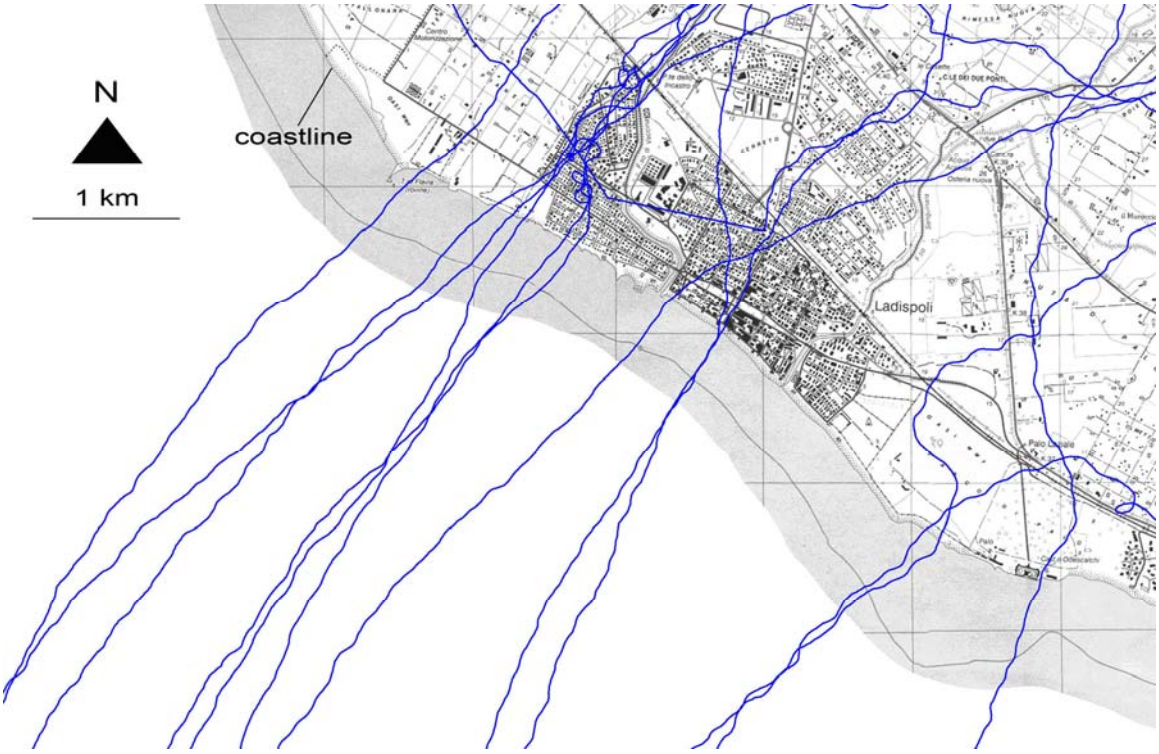

Fig. 2. Detail of Fig. 1B showing eight tracks crossing the coastline in correspondence of the town of Ladispoli and four tracks crossing over countryside and cultivated areas. The high density of conspicuous buildings makes towns the most evident feature of the coastline when looking from the sea.

Once over land, the majority of pigeons (28 out of 33) started to correct their home course and reorient towards the loft, after a variable path length. Some of them changed the home course gradually whereas others changed it abruptly. Only a few pigeons, four in each group, settled down soon after reaching the coast but this appeared not to be linked with the pattern of subsequent reorientation. On the contrary, five pigeons, three previously trained from the SE and two previously trained from the NW, continued to fly in the biased direction, two of them until their GPS batteries became exhausted (they, however, later returned to the loft), the others continued until the moment when they reversed their biased course.

The difference between the two groups in the average deviation from the beeline from the release site to the loft was highly significant in terms of direction (DEVlin, unpaired *t*-test: t_{31}= 7.614, *P*< 0.00001) whereas there was no difference in the magnitude of such deviation (ADlin, unpaired *t*-test: t_{31}= -0.26, *P*= 0.8). Moreover, no other flight parameters were influenced by the difference in previous directional training, indicating that the bias occurred primarily during the initial orientation step (*P*= 0.8 for TTfly; *P*= 0.1 for VE; *P*= 0.5 for HS; *P*= 0.5 for SI; *P*= 0.7 for ALT; *P*= 0.5 for TTrst; *P*= 0.9 for TNrst).

The influence of previous training was also assessed by measuring the average deviation of the path over the sea from the previous training direction. For the group previously trained from the NW it was 23deg. whereas for the group previously trained from the SE the deviation was 52 deg. This discrepancy is due to the fact that for the NW group, the previous training direction exactly corresponded at sea to the direction to the small promontory to the south on the coast whereas for the SE group, the previous

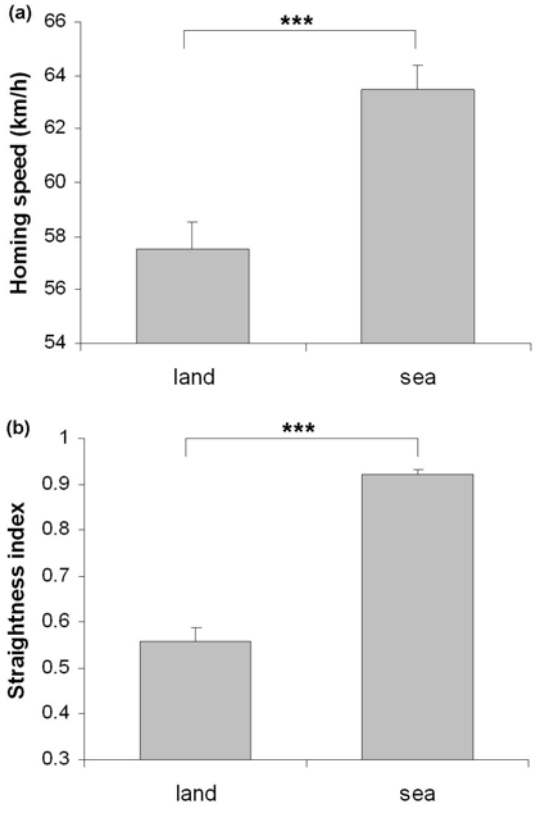

Fig. 3. (A) Average homing speed recorded by GPS and (B) straightness index during flight paths over sea and over land of the same tracks. When over sea, pigeons fly faster and straighter than when flying over land. Bars indicate means and s.e.m. ****P*<0.0001.

training direction was slightly beyond the promontory to the north. As a consequence, for this group, the coastline limited the deviation from the correct beeline, enhancing the deviation from previous training.

Comparisons of the tracks' segments over the sea with the segments over land showed that when flying over the sea, pigeons had faster and more direct flights than when over land (for HS, *t*-test: t_{31}= -4.42, *P*= 0.0001; for SI, Wilcoxon signed-ranks test: *T*= -4.94, *P*< 0.0001) (Fig. 3).

DISCUSSION

Our results showed a strong effect of previous directional training on initial orientation of pigeons released at sea. All pigeons had a strong tendency to fly with a bias in the direction from which they had been initially trained, even if there was an individual variability in the magnitude of deviation. Moreover, pigeons started to correct their direction only after reaching the coast, and showed high difference in flight behaviour over sea and over land.

The absence of proximal landmark cues over the sea obliged pigeons to rely primarily on their compass sense. The chosen sea release site was 20 km from the coast and equidistant to the shore for an angle of approximately 100 deg. symmetrically around the beeline from the release site to the loft. Thus, the directions chosen by the pigeons were not biased by simply choosing the shortest way to the coast. It is likely that, when released, pigeons saw the coastline, which probably accounts for the lack of initial flight paths pointing to the open sea. Moreover, the flight paths we recorded towards unknown coastal regions suggest that the birds were barely steering towards coastal landmarks, as highlighted by the fact that the majority of pigeons reached the coast over towns.

The observed flight paths over the sea appeared to reflect an individually variable compass setting between the tendency to rapidly approach the shore, the acquired training direction and the true home direction. In extreme cases, the birds either headed home almost perfectly or followed the previous training direction, respectively. On average, however, the flight direction over the sea appeared to reflect a mean between the true home direction and the previous training one. This is more evident in pigeons previously trained from the NW, for which the training direction corresponds to the direction of the small promontory to the south on the coast. Their average direction is exactly the mean between previous training and beeline to the loft.

As strict terrestrial birds, pigeons are neither able to swim nor float, hence they generally avoid flying over water surfaces; when forced to cross them, they choose the shortest way to the coast (Bonadonna et al., 1997; Wagner, 1972). However, on one hand, the choice of the release site (i.e. equidistant from the shore within 100 deg.) ruled out a directional choice based on the shortest distance to the shore. On the other hand, stress associated with flying over water may have led pigeons to reach the coast very rapidly and directly. This is reflected in the significantly higher speed and straightness of flight recorded over sea than over land, and is also supported by the fact that pigeons released at sea circled significantly less around the release point than pigeons released over land, even when from familiar locations (G.D'A., G.D'O. and H-P.L., unpublished data). Thus, the limited corrections of the flight directions over the sea may be related to the aversive component of this environment, giving previous experience a predominant role in the determination of bearings under these conditions.

Pigeons reached land over completely unknown territories, as birds trained in the north reached land south of the loft and *vice versa*. Once they had reached the coast, only a few individuals continued to fly in the direction of previous training whereas most of them (28 out of 33) were able to correct their course more or less gradually. Such changes in flight direction suggest that the acquired directional training was no longer a decisive factor, once they were over land, in homeward orientation.

Wallraff has hypothesized that the less information available for orientation the more pigeons rely on previous flown direction (Wallraff, 1974). From our data it is not possible to conclude whether the terrestrial environment contains more information for a correct orientation or whether pigeons could have already determined the correct loft position over the sea but preferred to first reach the coast rapidly and follow the homeward direction afterwards. Once over land, pigeons appeared attracted by landscape features, thus increasing path tortuosity and decreasing flight speed. Lau and colleagues demonstrated that pigeons are generally attracted by territories with higher densities of edges, which are considered a relevant feature characterizing landscapes (Lau et al., 2006). Over such kind of territories, pigeons' flights show higher levels of entropy, i.e. an increase in directional and orientation changes. Therefore, the attraction by landmarks could distract pigeons from the chosen home direction, inducing them to frequently reorient and gradually redirecting towards the correct home course.

At the same time, some pigeons, in particular those that continued to fly with a bias in the training direction after crossing the coastline, turned abruptly, changing their route by

90 deg. to 180 deg. We can suppose that pigeons inverted their route most probably when the map mechanism gave them the information that the home loft was actually in the opposite direction (Wallraff, 1991).

An alternative explanation could be the existence of a sense of distance, perhaps an odometric memory as found in other species homing (Wittlinger et al., 2006). This sense, possibly acquired with repeated release experience from the same homing distance, may provide pigeons with an indication of the approximate flight distance to the home loft, leading them to reorient once this distance has been covered. In our study, most birds changed their orientation soon after reaching the coast, which was only at a slightly shorter distance from release point than previously experienced homing distance. This makes it impossible at present to discriminate between the two factors that would necessitate more specific experiments, such as releases of pigeons previously trained from different distances. Corrections after an expected flight distance, however, are frequently observed by GPS tracking of highly trained pigeons [for examples, see the flight tracks of the training flights (Fig. 1A), and also tracks in Lipp et al. (Lipp et al., 2004)].

In conclusion, releases over the sea can reveal orientation behaviour of pigeons with particular clarity, as flight paths are less confounded by topographical attractors. Our results indicate that previous flight experience resulted in a conflict between acquired directional experience and setting off the compass direction from an unknown release site, expressed over the sea by the motivation of reaching the shore as fast as possible. Once they had reached the coast, pigeons were able to correct their biased orientation and redirect homewards. Further investigations would be necessary to elucidate the relative role of the various potential mechanisms operating such correction, including the map mechanism, the influence of landmarks or the presence of a sense of distance.

LIST OF ABBREVIATIONS

ADlin average distance to beeline between release site and loft

ALT flight altitude

D beeline distance from starting point to the goal

DEVlin direction of deviation from the beeline between release site and loft

HS homing speed

L total path length flown

SI straightness index

TNrst total number of rests

TTfly total flying time

TTrst total duration of rests

VE vanishing error

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Chapter 3

Waiting duration before being released increases the motivation to home in homing pigeons (*Columba livia*).

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Waiting duration before being released increases the motivation to home in homing pigeons (*Columba livia*)

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ABSTRACT

When performing homing experiments, pigeons have to wait in a transport box for a certain time before being released individually and hence perceive departure of companions. Quite often, the last pigeons disappear straightforward from the release site. The question is whether this reflects improved orientation because of prolonged exposure to the release place, or whether it reflects increased homing motivation. By releasing pigeons from a familiar site, we investigated the effects of the time spent at the release site on homing performance, recording pigeons' flights with GPS loggers. Our results show that, despite individual peculiarities of flight patterns, the waiting duration at release site had a positive effect on homing speed and time, and reduced the time spent circling around the release point. On the other hand, the overall path efficiency as derived from GPS tracking was not influenced. These results suggest that long waiting before release improves homing performance and this is related not only to increased navigational abilities but also increased homing motivation.

INTRODUCTION

The homing pigeon (*Columba livia*) has been intensively used as a model species to study orientation and navigational capabilities of birds. In spite of this great interest, however, only a few studies dealt directly with the possible influences of birds' motivation on homing performance (i.e. Del Seppia et al., 1996). On the other hand, pigeons are commonly used for racing by fanciers who have successfully developed differential strategies (i.e. food reward, manipulations of the brood or the clutch) aimed at improving the motivation to home when displaced and the homing performance during competitions. However, despite this anecdotal knowledge, a systematic scientific research on this topic is missing. In the scientific literature, this topic has not received attention and only recently has been considered, although mostly when discussing results (Biro et al., 2002; Dell'Ariccia et al., 2008; Schiffner and Wiltschko, 2009).

To perform homing experiments, pigeons are taken from their lofts and transported to a determined location in wicker baskets, where they stay until the moment they are released, generally individually. Immediately after release, most pigeons first circle around the release sites in a radius of about 1 km for some time before choosing a flight direction (Matthews, 1951; Schiffner and Wiltschko, 2009). We consistently observed that during a series of releases, the last released pigeons headed home more rapidly and circled less around the release site than the first ones. Such observations suggested an influence of the duration of waiting at the release site on subsequent homing.

Although it seems clear that circling time has a social and motivational component (Biro et al., 2002; Dell'Ariccia et al., 2008; Schiffner and Wiltschko, 2009; Wiltschko et al., 2007), the rapid vanishing of late-released birds could reflect improved adaptation to local navigation cues. Using GPS tracking permits to assess both the flight behaviour around the release site and the navigational efficiency during the entire flight home. Thus, if prolonged waiting improves navigational efficiency, one would expect that late-

released birds navigate more efficiently during both the early flight segments around the release site and during the entire path homewards. Should the rapid vanishing of long-waiting birds reflect their motivational status, one ought to expect efficient orientation and fast vanishing primarily around release site, and higher flight speed also during the rest of the homeward journey. At the same time, purely navigational parameters such as path efficiency and distance to the beeline should be comparable with the ones of the early starters.

METHODS

For this study, we re-analyzed a set of GPS tracks from our database. We selected a series of 16 releases that took place between November 2004 and April 2005 from the release site Santa Severa (11.98° N; 42.03° E), 27 km NW of the loft. During this series only pigeons without any experimental manipulation that could alter homing capabilities were released, all individually. Moreover, it included only pigeons already very familiar with the site. High familiarity allows better assessing the influence of waiting duration, because of the reduced orientation uncertainty after repeated releases from the same site (Graue, 1965; Wallraff, 2005).

A total number of 21 homing pigeons of both sexes, 1-4 years old, participated in the selected releases. All pigeons were housed in the facilities of the University of Zurich at Testa di Lepre, Italy, 25 km NW of Rome (12.28° N; 41.93° E), where they were habitually allowed to fly freely outside the lofts and they underwent regular training. They carried a PVC dummy of the size and weight of the GPS loggers (20g, www.technosmart.eu), to habituate them to fly with the load. Dummies and loggers were attached by means of an adhesive Velcro strip glued on the feathers on the back of the pigeon with a procedure already described in Dell'Ariccia et al. (2008).

In the 16 releases, a different number of pigeons were used (2 to 13; mean ± s.e.m.: 6.94 ± 0.89). Every pigeon participated in 2 to 10 releases (mean ± s.e.m.: 5.75 ± 0.64) and the release order was random each time. All releases took place under at least partially sunny conditions, winds being absent or weak.

To have a standard method to measure the waiting duration of each pigeon, we set as zero the time of the first pigeon released, and then we counted the waiting time of successive pigeons until the moment of their release as the number of minutes and seconds passed from the first. During waiting, pigeons were in their habitual transport wicker basket. Prior to release, we substituted the PVC dummy with the GPS logger, and we placed the pigeon in a small starting crate that was opened after 2–3 min. Birds were placed in the starting crate and released in intervals of 5-10 min, only after the former pigeon had disappeared from sight. From this crate only the sky but not the surrounding landscape was visible. The loggers took one positional fix every second, and then stored the data. Further technical information can be found in Biro et al. (2002) and Lipp et al. (2004).

Qualitative and quantitative analyses of individual tracks were run with WINTRACK freeware (www.dpwolfer.ch/wintrack) (Steiner et al., 2000; Wolfer et al., 2001). This extracted the following variables: homing speed (HS: average speed recorded by GPS logger during flight, excluding measures of speed of less than 5 km/h), flight altitude (ALT), flying time (FT), path (Pbeg) and time (Tbeg) till leaving the start zone (defined, respectively, as the distance flown and the time spent before leaving a circle of 1 km radius about the release point) and average track distance to the beeline between the release site and the loft (ADlin). We calculated also the straightness index (SI) for each track as D/L, in which D is the beeline distance from the starting point to the goal and L is the total path length flown (Benhamou, 2004). This is a scale independent measure

and, given the highly detailed path reconstruction at 1 fix/sec, a reliable estimator of the efficiency of the orientation process.

The variables obtained by Wintrack and the straightness index were subsequently statistically analyzed using General Linear Models in the STATISTICA package (Version 7.0, StatSoft, Inc. 2004, Tulsa, OK, USA). In each model, we included “release day” and “individual” as random factors, the “waiting duration” as a covariate and the interaction between “individual” and “waiting duration” to evaluate whether individual pigeons behaved in a similar way regardless of the waiting duration. This also allowed us to correct for pseudoreplication due to the dependence of measures on the same individual. The interaction had been removed from the models when non significant and the analyses were repeated according to Engqvist (2005).

The normality of distribution was checked using the Kolmogorov-Smirnov test and the Shapiro-Wilk’s test. The variables that did not meet the normality criterion were log-transformed (ADlin, FT) or transformed using the reciprocal (ALT, Tbeg, Pbeg). The way of transforming the data was chosen as that which better improved the normality of the distribution. Coefficients of partial correlation were calculated when we had found a significant covariation.

RESULTS

Overall we analyzed 111 pigeons’ tracks. Figure 1 illustrates an example of GPS tracks obtained from the first two and last two pigeons released, after about one and a half hours, during the same release day (Fig 1a, c) and the tracks recorded from the same pigeon during different days, when released after different waiting durations, as the first one (blue) or after about 30’ waiting (red) (Fig 1b, d). It appears evident that the blue tracks of early released pigeons are more tortuous around the release site (Fig. 1c, d) while there is no difference in directedness of flight and deviation from the beeline in the

subsequent flight path (Fig. 1a, b). Different release days and different pigeons showed not always this same clear pattern, for this reason we inserted these two factors in our analyses to statistically assess their influence on the final output.

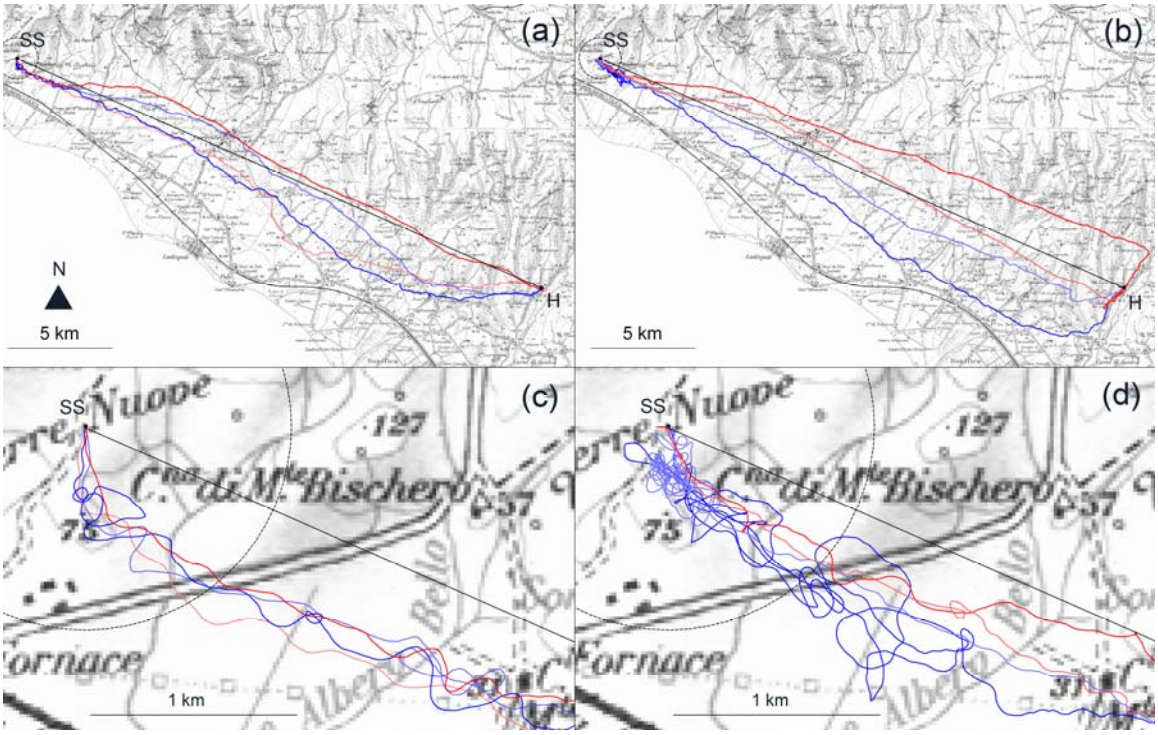

Figure 1. Examples of GPS tracks obtained (a) during the same release day from firsts (blue) and lasts (red) released pigeons and (b) from the same pigeon, during different release days, when released as first one (blue) or after about 30' waiting (red). (c) and (d) Detail of the release site of 1a and 1b, respectively, showing the increased circling of early released birds. The circle shows the start zone of 1 km radius. SS = release site. H = home loft.

The pattern of covariation between the waiting duration and each of the flight variables was similar across pigeons (individual x waiting time: all p-values ≥ 0.25). Therefore, the interactions were removed and the analyses were repeated (see table 1 for a list of the final models). All the examined variables showed significant differences between individuals, indicating that homing performance was influenced by individual behavioural tendencies. Despite these individual differences in performance, our analyses show a

positive covariation between the waiting duration and the homing speed (partial r = 0.48; Fig. 2a), a negative covariation between the waiting duration and the log-transformed total flying time (partial r = -0.46; Fig. 2b), and a slight positive covariation between the waiting duration and the reciprocal of both time (partial r = 0.22) and path (partial r = 0.22) to leave the start zone (a circle of 1 km radius about the release point) (Tab. 1). The straightness index, the flight altitude and the average deviation from the beeline did not significantly covary with the waiting duration (Tab. 1). The homing speed and flight altitude differed significantly between different releases, indicating an influence of the release day on such parameters (Tab. 1).

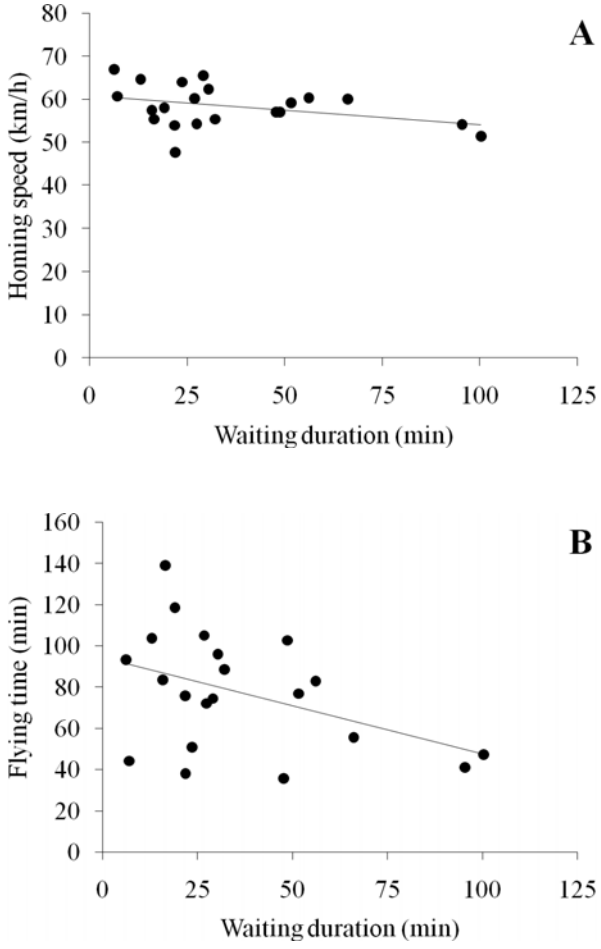

Figure 2. The longer pigeons waited in the transport basket, the quicker they flew (A) and the shorter they homed (B). Mean-pigeon values are shown (n = 21) to correct for pseudoreplication.

	Circling time (Tbeg)		Circling path (Pbeg)		Homing speed (HS)		Flying time (FT)		Straightness index (SI)		Deviation from beeline (ADlin)		Flight altitude (ALT)	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
release day	1.261	0.24871	1.271	0.24160	26.538	**0.00000**	1.653	0.07999	0.932	0.53356	1.592	0.09631	5.516	**0.00000**
individual	2.226	**0.00765**	2.494	**0.00270**	4.119	**0.00001**	2.638	**0.00154**	3.031	**0.00033**	3.471	**0.00006**	6.443	**0.00000**
waiting duration	3.901	**0.05195**	3.685	**0.05869**	17.694	**0.00007**	20.350	**0.00002**	2.133	0.14833	2.017	0.15972	2.441	0.12243

Table 1. The results of the GLM test to examine the influence of "release day", "individual" and "waiting duration" on the different variables. Significant values are highlighted in bold.

DISCUSSION

Our results showed that the waiting duration before being released has an influence on homing behaviour, even if there are individual tendencies in homing performances blurring this effect.

Individual behaviour influenced all examined variables, underlining that every pigeon had his own homing characteristics. This could reflect both different orientation capabilities and different motivation to home. Wallraff (1994) attributed individual variability in orientation to stochastic noise and not to consistent differences among individuals. According to his study, a high homing speed in one flight does not generally indicate high performance level of the individual bird but the existence of other factors influencing orientation even if he did not identify the nature of such factors. In our case, individual differences in homing behaviour could also reflect the development of individual stereotyped routes, as it often happens when pigeons are repeatedly released from the same release site (Biro et al., 2004).

Despite these individual differences, our results show an effect of waiting duration at the release site on homing speed, the time and path length till leaving the start zone, and the total homing time, while the other navigational variables were not influenced. This suggests that there is no improving in navigational capabilities: flight efficiency and the

average deviation from the beeline were not influenced by the waiting duration nor was the flight altitude. On the contrary, the longer a pigeon waited the faster it flew home and, consequently, the shorter was his homing time. A faster homing speed without an improvement in flight efficiency suggests an influence of homing motivation that appears to be enhanced by the wait.

Also the analysis of the initial phase of homing flight supports the motivation hypothesis. Pigeons, when released, spent some time flying around the release point, or just nearby, before heading home (Matthews, 1951; Schiffner and Wiltschko, 2009). This initial phase of flight has been mainly explained as a gathering of navigational information at the site (Biro et al., 2002; Spott, 1993) or a technical preparation to the flight (Schiffner and Wiltschko, 2009); therefore we can suppose that this can not reduce below a certain threshold. However, the reduced time and flight path in the start zone evidenced in our results, even not excluding the orientation function, are in accordance with the hypothesis of a motivational and social component influencing this initial phase (Biro et al., 2002; Dell'Ariccia et al., 2008; Schiffner and Wiltschko, 2009; Wiltschko et al., 2007). The time spent in the start zone is dependent on the flight speed, which resulted increased by waiting, but also on the flight path length in this zone that resulted reduced, indicating that pigeons head earlier towards home. This could reflect an increased will to reach the home loft after the wait in the basket or a decreased circling to look for flight companions. This last point being even truer for the very last pigeon of the release: it remained alone in the basket so it was probably aware that no other pigeons can be released to join him on his homeward trip.

Since no particular landscape preview at release site was given to our pigeons, we can exclude the possibility that was the recognition of familiar landmarks to account for the better performance at release site, as found in previous studies in which pigeons allowed to preview the landscape at release site circled less around the release area than

pigeons to which such preview was denied (Biro et al., 2002; Braithwaite and Newman, 1994). Moreover, in our case the homing speed during the whole flight was influenced, increasing gradually with the increment of waiting duration.

A different explanation could be that pigeons waiting for longer have more time to get and process olfactory and magnetic information. This could have helped pigeons at the moment of release to a faster depart. The idea should be directly verified, however we can suppose that a pigeon better informed about his position and the direction to take will head home not only faster but also more efficiently in respect to a pigeon that had gathered less information. This we found to be the case during the initial phase but not during the rest of the flight, suggesting that overall navigation was not influenced. To better explore this topic, future studies could also investigate if there are differences in performance when the whole group of pigeons wait for a long time before the first pigeon is released. Such studies could help to assess if the waiting duration alone has an influence on subsequent homing or if there are also other factors such as the hearing of previous companions being released.

The day of the release affected only the homing speed and the flight altitude. This could be a consequence of slightly different meteorological conditions, such as the intensity and direction of the wind and the visibility over different days. These environmental factors however did not preclude a significant effect of the waiting duration.

In conclusion, our study shows that navigation behaviour can be influenced by homing motivation, especially during the initial phase. The waiting duration before being released can be an enhancer for homing motivation. This should be taken into account during homing pigeons experiments even if further studies are needed to better understand and evaluate the role of motivation in homing.

LIST OF ABBREVIATIONS

ADlin average distance to beeline between release site and loft

ALT flight altitude

D beeline distance from starting point to the goal

FT flight time

HS homing speed

L total path length flown

Pbeg path length to leave the start zone

SI straightness index

Tbeg time to leave the start zone

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Chapter 4

Additional unpublished studies, data and observations

Additional unpublished studies, data and observations

Monocular viewing pigeons use longitudinal landmarks to facilitate group cohesion

The importance of landmarks for flock-flying pigeons has been evidenced in another experiment we performed in which groups of pigeons were released with monocular occlusions. This study was aimed to test the effects of cerebral lateralization in group navigation, asking whether pigeons use the left or the right hemisphere for cohesion during the flight (as visual input is conveyed to the contralateral hemisphere). In case of a hemispheric superiority for group cohesion in flight, one would have expected the group with the superior hemisphere to keep together, and the group with the non-specialized hemisphere to split up rapidly.

We repeatedly released two groups of six pigeons each, from two different locations, alternatively closing the right, the left or none of the eyes. When released with monocular occlusion, all the group members had occluded the same eye.

Both groups with left- or right-eye occlusion split soon and about equally. Therefore, the experiment did not provide evidence for hemispheric specialization.

However, from the tracks it was possible to see that conspicuous longitudinal landmarks pointing home allowed visually impaired pigeons to keep group cohesion, as seen in figure 5. The release site South-East to the loft, Castel Romano, is in the middle of a big plain that continues northwards to the loft. In this condition pigeons with impaired vision were completely unable to keep group cohesion and split, one after the other, relatively soon after release (the longer group track is about 5 km). The splitting happened in both groups when released from this site and in both conditions of left or right eye closed (Fig. 5a). On the contrary, the release site North-West to the loft, Santa Severa, is at approximately a middle distance between the coastline and a chain of low mountains, which develop North-South in a parallel course to the loft direction. This particular geographical location, together with the fact that from this site pigeons generally show high scores of road following (Lipp et al. 2004; Dell'Ariccia et al. 2008), probably accounted for a smaller deviation from the beeline to the loft in respect to Castel Romano (Fig. 5b). Moreover, important to notice is that in the condition with the left eye open/right eye closed both groups, released on different days, flew in a cohesive flock for about 13 km until the same point (marked by the arrow in Fig. 5b) where they

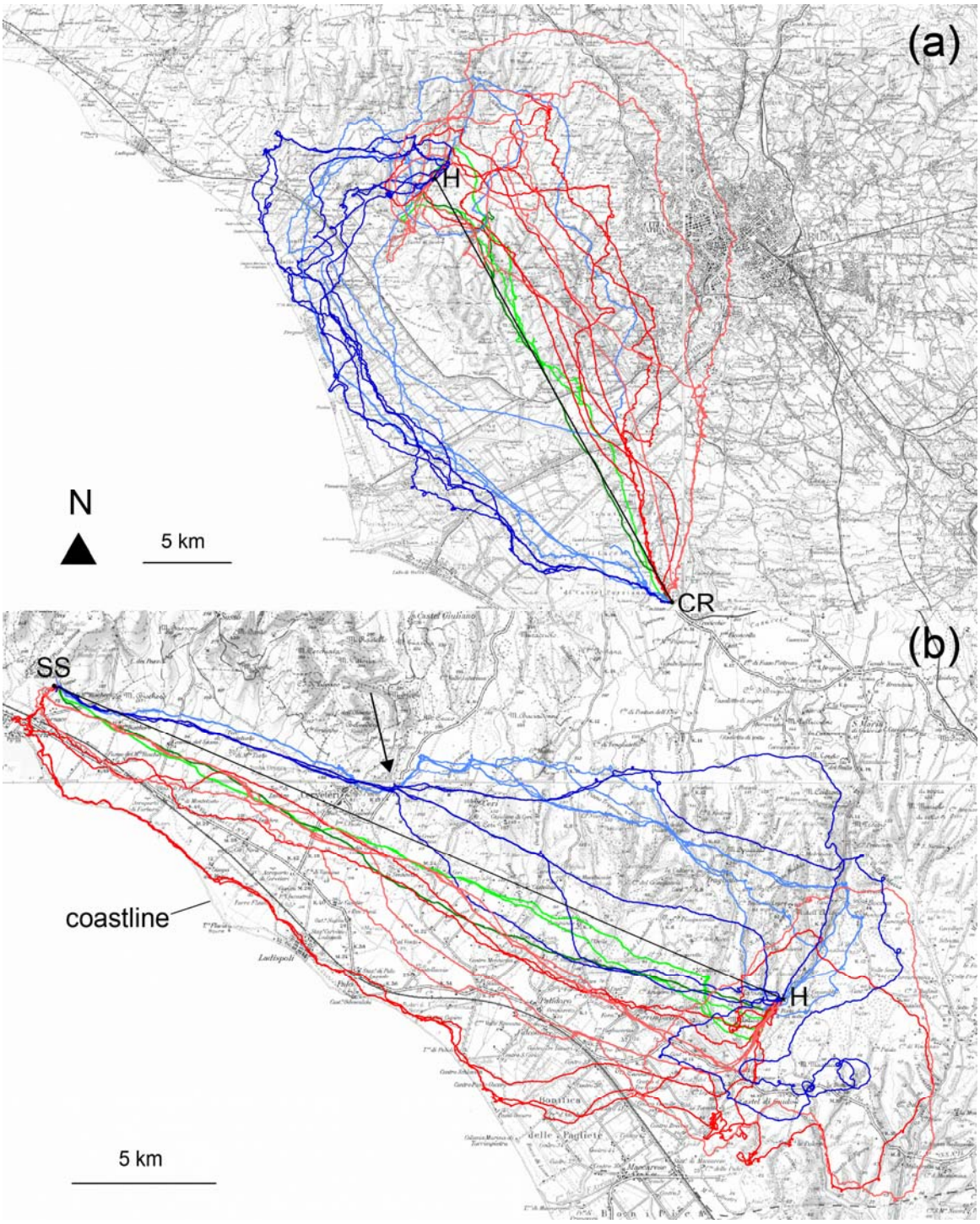

Figure 5. Flight tracks of monocular viewing groups. Two groups of six pigeons were released in three different conditions: all group members with the right eye open/left eye closed (red tracks), all with the left eye open/right eye closed (blue tracks) or as controls with both eye open (green tracks). For each color, the dark version is group A, the light version is group B. (a) The releases were performed each three days from the release site Castel Romano (CR): the first both groups as controls; the second, group A with left open/right closed and group B with right open/left closed and the third, group A with right open/left closed and group B with left open/right closed. (b) After other three days the same sequence of releases was repeated from the release site Santa Severa (SS). The arrow shows the end of the hill chain.

suddenly split. Looking at the map, we found that this point corresponds to the end of the mountain chain and the beginning of a plain (the same that continues southwards until Castel Romano and over). In the other condition, with the right eye open/left eye closed, one of the two groups, at the moment of release, deviated until the coastline and started to fly exactly over it. This flock rested cohesive until it continued to overfly the coastline and split only when obliged to leave it and turn towards the loft.

These observations again suggest an important influence of landscape features for aligning flight directions. Such aligning obviously facilitates group cohesion when pigeons are released with monocular viewing.

How many leaders in a flock?

Our results on flock flights suggested the absence of a leader bird with better navigational abilities, leading the companions homeward. Since the precision of the GPS loggers used did not allow testing this hypothesis directly, we found only indirect evidence for this, checking for every release the rank order of the pigeons according to their performance. In the case of a typical leader dictating speed and direction of the flock, the leader bird should have consistent performance in individual and group flights. However, we failed to identify a pigeon with constant superior performance.

In this case too, the use of a new technology allowed us to answer questions left open. We applied a new and lighter GPS device to 24 groups of four-to-six pigeons. These devices are capable to record four positional fixes per second with a precision of 4 metres in 95% of fixes, reducing by four times the temporal error of the previous GPS model. This higher resolution allowed us to look directly into the group dynamics during flight. The preliminary results investigating the leading position inside the group confirm the previous findings of the absence of a fixed leader and evidence a high sharing of the leading position. Figure 6 shows an example of two different tracks with different percentages of leadership sharing. In some of the flights, the pigeons continuously alternate while in other flights one group member showed higher values with respect to other companions. This different pattern, however, varied both between different groups and between different flights of the same group, without any correlation with sex, age and experience of the pigeons nor with the familiarity and distance of the release site.

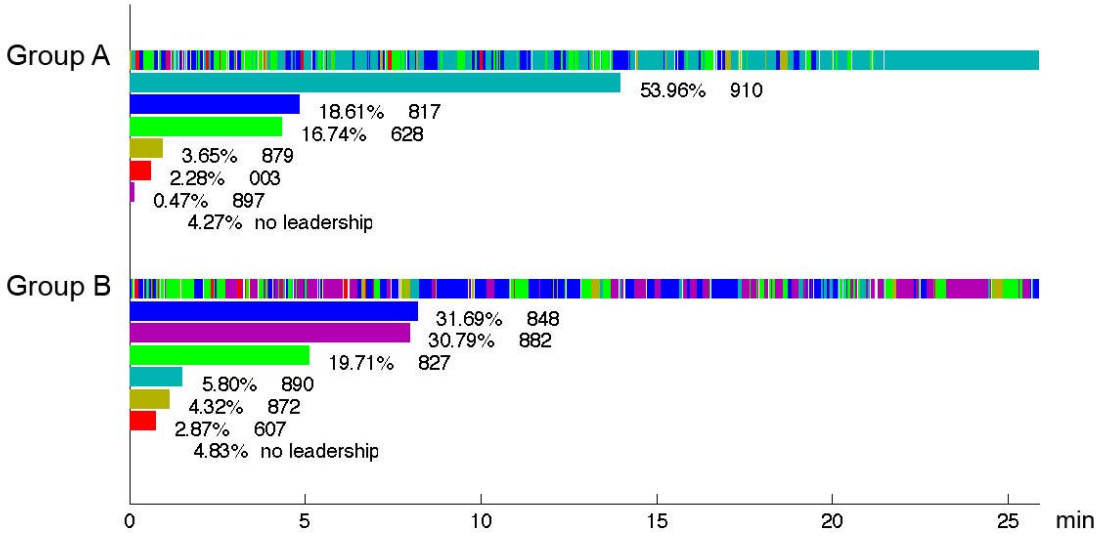

Figure 6. Minutes and percentages of time spent at the leading position of two groups of six pigeons released the same day from the release site Santa Severa, 27 km NW to the loft. For each group, the first band show the leader pigeon during the entire flight respect to the time spent from release (on the abscissa). The subsequent six bands represent the cumulative scores with the relative percentages for the individual pigeons composing the flock.

Idiosyncratic behaviour

A last point emerged from our results concerns the individual differences in homing behaviour. When performing homing experiments, a more or less large group of pigeons is used, their behaviour measured in the terms asked by the experiment and the data pooled to perform statistics and obtain an answer to the original question. This approach assumes that, in average, all pigeons behave similarly and that variations of measurements occur stochastically. In Chapter 3, we reported that there were statistically different individual tendencies in homing behaviour subsequent to different waiting durations for all the measured variables. Taking the circling behaviour as example, this means that certain individuals circled systematically more than others. This was true also with an equal waiting duration.

During the numerous releases performed for this thesis, it was possible to observe and record highly idiosyncratic individual behaviours. For example, when releasing pigeons from Santa Severa (27 km NW to the loft) there was one pigeon that systematically flew to the nearby castle (< 2 km), spent there some time (from a few minutes to up to more than one hour) and, only after this time, headed home (Fig. 7a).

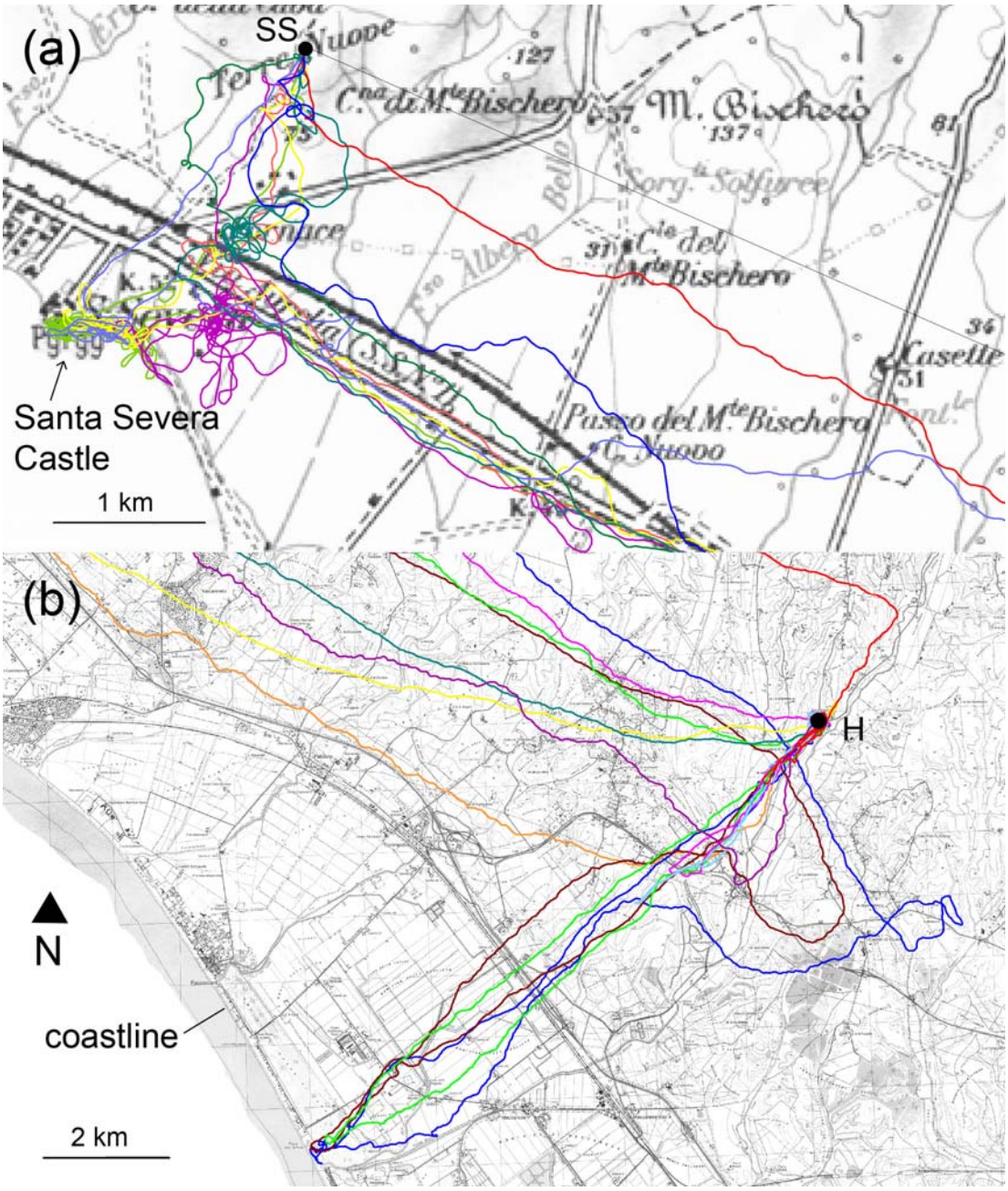

Figure 7. Examples of tracks showing particular individual behaviours. (a) Pigeon n°100 flying to the Santa Severa Castle every time after being released from this site (SS) before heading home. (b) Pigeon n°823 flying towards the coast after homing and once directly from a free flight at the loft (H). In both cases, different colours correspond to tracks recorded in different days from the same pigeon.

Another pigeon flew regularly towards the coast after homing from a release site (Fig. 7b). Such idiosyncrasies have already been reported sometimes before and justified as a different use or weight of available orientation cues by different individual pigeons, even when raised and trained under identical conditions (Walcott 2005); or as induced

by the social motivation of joining local living flock of feral pigeons (Wiltschko et al. 2007). When working with large numbers of pigeons, these individual idiosyncrasies disappear in the group data. It is only by studying individuals that they become apparent.

While rarely done today in navigation research, studying individual behaviours may be helpful to understand the biology of personality traits and habit forming. For example, it is generally assumed that pigeons like to fly over unfamiliar territory, but many observation suggest that at least some birds are afraid of this and hesitate (or refuse) to leave the release place, particularly under conditions of reduced visibility. Therefore, it appears necessary to assess more systematically idiosyncrasies and motivational status of the pigeons, as this may change the interpretation of results.

The neurologgers: a new technology for old questions

The advances in technology are something that, fortunately, never stops. So, after the improvement in navigation studies had with GPS loggers another important step has been done with the developing of miniature EEG recorders, the neurologgers, that give the possibility to record the electrical brain activity of birds during flight and then to merge it with GPS tracks (Vyssotski et al. 2006). One of the most important recent finding is that there is a peak in EEG activation in the middle frequencies band (12-60 Hz) in correspondence of familiar landmarks and when pigeons released over the sea arrive to cross the coastline (Annex 1, Vyssotski et al. *In press*).

Over the sea there is the peculiarity that there are no visual cues to orient and so the pigeons have to rely on other mechanisms. This particular condition evidenced the deviating effect of directional experience, because birds departed directly towards the coast in the direction they considered homeward (Chapter 2, Dell'Ariccia et al. 2009). Only after reaching the coast they started to reset their compass and correct their direction. The significant increase in path tortuosity suggested an influence of the presence of landmarks, at least as distracting cue that induced frequent reorientations. These findings have been confirmed and integrated by successive sea releases in which birds were equipped with neurologgers. The EEG power in the range of 12-60 Hz decreased linearly with the distance from the release point up to approximately 1 km from the coast, where it started to rise again (Fig. 2a in Annex 1). The EEG activation over land was higher than over the sea, moreover the same sharp peak observed in correspondence of the coastline was also observed when crossing familiar landmarks, indicating that a generalized response exists when pigeons fly over familiar objects.

Such a response was present also in flock flying birds but in this case the increase of EEG power subsequent to landmark crossing was lower compared to when pigeons flew alone (Fig. 4 in Annex 1). A possible explanation is that when flying in flocks, pigeons must divert visual attention to their companions in order to keep group cohesion. This new finding well corresponds to the previous one that flocks rely less on longitudinal landmarks shifting the balance between orientation strategies towards a compass navigation and that is this shift between different strategies to account for the most part of the improving in performance of flocks in respect to individual flyers (Chapter 1, Dell'Ariccia et al. 2008).

Discussion and Conclusions

Taken together, the results of this study show that all the investigated behavioural factors had a noteworthy influence on homing performance. Flying in flock improves considerably homing performances in terms of navigational accuracy, speed, and motivation, even in releases from highly familiar release sites. Previous directional training biases the initial orientation when pigeons are released at a new location. Finally, the waiting duration before being released enhances the motivation to home; the flight speed is increased while the other navigational parameters remained unchanged.

The use of GPS loggers allowed a much better analysis of homing. The GPS loggers directly measured at each fix per second the coordinates and the altitude. The recording of these variables along the entire path home allowed reconstructing at high resolution the birds' behaviour till the home loft and to extract, using an appropriate software (WINTRACK freeware, Wolfer et al. 2001), other variables such as the flight speed, the time and path length in the start zone, the number and duration of rests, the actual path length flown, the path linearity and the deviation from the beeline to the loft, the path length along the main roads, etc. These parameters gave us an enormous amount of information that was not available in all previous studies.

Flock flying pigeons headed directly home, showing less circling at the release site, higher homing speed, no rests, higher path efficiency and lower path length along roads in respect to individual flying pigeons. GPS tracking evidences that pigeons can dynamically shift between different coexisting strategies: individually flying pigeons show a greater reliance on topographical features for homing, keeping habitual home routes, while flocks tend to adopt a compass-based navigation.

Previous flight experience resulted in a conflict between acquired directional experience and setting off the compass direction from an unknown release site, evidenced by pigeons' tracks over the sea varying between the direction of previous training and the correct homeward direction. An important aspect was the potential of sea releases and the associated characteristic of the absence of landmarks that can reveal orientation behaviour of pigeons with particular clarity, as flight paths are less confounded by topographical attractors. A fundamental aspect shown by GPS loggers was that pigeons, motivated to reach the shore as fast as possible, started to correct

their direction homewards only after reaching the coast, and showed high difference in flight behaviour over sea and over land.

The waiting duration before being released decreased the flight path length and time spent circling at the start zone before departure. GPS tracking showed that the flight speed during the entire trip home linearly increased with waiting duration, while the other navigational parameters, such as path efficiency and the deviation from the beeline to the loft remained unvaried suggesting that there was no improving in navigation capabilities. A faster homing speed without an improvement in flight efficiency suggests an influence of homing motivation that appears to be enhanced by the wait.

Behavioural factors are not independent

Concerning the examined behavioural factors, an important point is that all factors are not independent of each other and they interact to give the final observed homing performance (see also Fig. 4). One of the main positive observed effects of group releases was the increased motivation to home of pigeons. Pigeons are a social species and, except when forced to fly individually for homing studies, they usually prefer to fly in groups. It is, therefore, not surprising that they are more willing to fly when allowed to do it with companions than alone. This can also be observed from the reduced circling time at the release site of groups. As already discussed in Chapter 3 on homing motivation, the time pigeons spend circling is dependent on several factors. There is an orientational component: birds get information about where they are and where they have to fly (Biro et al. 2002; Schiffner and Wiltschko 2009). This of course is predominant at unknown release sites. Additionally to this, there is a physical preparation to the flight as i.e. gathering altitude (Schiffner and Wiltschko 2009). Finally, it is more and more recognized the social component of circling (Wiltschko et al. 2007; Dell'Ariccia et al. 2008; Schiffner and Wiltschko 2009): an individually released pigeon start to circle to look around for previously released companions and/or to wait for the next one to be released. Very often a pigeon circling for protracted time (more than 10 minutes) head homeward as soon as another bird is released. The reduced circling of groups we observed is, therefore, due only in part to increased homing capabilities as predicted by models on group navigation (Bergman and Donner 1964; Simons 2004; Conradt and Roper 2005) but mainly to the increased motivation of flocks to home.

The importance of GPS loggers and new technologies

The use of GPS loggers was fundamental to obtain our results. GPS loggers, recording at high resolution the entire pigeons' path home, gave the opportunity to collect a big amount of information on pigeons' behaviour that was not even imaginable before. Moreover, in all our experiments the use of GPS loggers allowed to find something more than the simple answer to the main experimental question.

1. In the first experiment, comparing the homing performance of individual versus flock flyers (Chapter 1), the GPS showed an increment in homing performance of flocks concerning both homing speed and directness, and also the circling at release site, the number of stops and the path along the main roads pointing home. Moreover, the use of GPS loggers evidenced that the better performance of flocks was not only due to their improved orientation capabilities in respect to individuals, as predicted by models, but mainly to a change in navigational strategy, from landmark following to a compass based navigation. This showed for the first time that pigeons not only can rely on different orientation cues but that they can use them alternatively and freely choose between them.
2. In the experiment aimed to understand the effect of directional training on orientation (Chapter 2), we highlighted that previous training had a strong influence on the initial orientation of pigeons that resulted in a conflict between the direction of previous training and the real home direction. In addition, the possibility to trace the entire flight tracks allowed seeing that pigeons started to correct their home course only after reaching the coast, suggesting an influence of landmarks in such a process, and that they showed different patterns for reorientation; some of them reoriented gradually while others turned abruptly, changing their route by 90° to 180°.
3. Measuring the effects of the waiting duration at the release site on subsequent homing behaviour (Chapter 3), the use of GPS loggers revealed that the increase in performance after longer waiting was circumscribed to the release site, where pigeons circled for shorter times. On the contrary, during the rest of the journey the performance did not change but for the speed, suggesting an influence of an increased motivation to home for the birds that waited the most.
4. GPS tracking displayed the importance of landmarks in monocular viewing pigeons to keep group cohesion (Chapter 4) enabling to precisely determine the locations of group splitting.

5. Idiosyncratic behaviours would not have been identifiable without the GPS recording of flight paths (Chapter 4).
6. The development of new GPS loggers with a higher temporal resolution allowed directly investigating the dynamics during group flights, with particular attention to the leading position (Chapter 4). This evidenced that there is a high sharing of leadership in flocks and that almost all group members participate in the determination of the home course.
7. Finally, the invention of neurologgers to combine with GPS loggers gave a fundamental increase in the understanding of pigeons' homing mechanisms (Annex 1). The possibility to examine the brain activity synchronized to the GPS loggers allowed also confirming the findings of previous works on flocks' behaviour in respect to landmarks and the different behaviour during flights over the sea and over land (Chapter 4).

All these findings added some fundamental information to the knowledge and understanding of homing behaviour. Moreover, all these created new questions and opened new perspectives in homing research.

However, what is also important to notice is that the use of this kind of technology has an intrinsic potential bias given by the fact that the loggers, GPS or neurologgers, need to be recollected to download the data. This implies that the only available data are those from pigeons able to return to the loft. In the experiments reported in this thesis we only recorded and measured pigeons' behaviour as an observation of it. Pigeons were not manipulated in the way to impair their homing abilities for a comparison to control birds and only a very small number of pigeons were lost during the sea releases. When pigeons are seriously impaired in their homing capabilities, as for example in experiments of anosmia from new release sites, a big amount of losses often occur. In this last case the collected GPS data have to be weighted and correctly valued in the way to not misinterpretate the results.

Conclusions

In conclusion, this study added some important information to the study of homing behaviour recognizing for the first time the big importance of behavioural factors influencing orientation and navigation. The orientation mechanisms alone, i.e. the position of the sun or odours, do not explain completely what is observed during experiments because a number of interacting factors have a strong influence, changing the course of navigation. Moreover, our results showed that pigeons are able to use and choose between different homing strategies according to their condition. Pigeons are strongly influenced by the possibility to fly in a group and by previous training. Also the duration of waiting at release site is important, enhancing homing motivation. This study again confirmed the importance of landmarks for navigation, also thanks to the use of neurologgers, and for keeping group cohesion in monocular viewing pigeons. Our results, together with the continuous development of new technologies that allow having more detailed information, open new perspectives in the study of homing behaviour, orientation and navigation. The behavioural factors affecting homing and navigation need to be fully recognized and assessed. As an example, the importance of the motivation to home that has been previously ignored in scientific literature and, even if difficult to isolate and evaluate as a source of variability, must be included among the cues playing a role in the orientation process.

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Annex 1

EEG responses to visual landmarks in flying pigeons

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EEG responses to visual landmarks in flying pigeons

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ABSTRACT

Background: GPS analysis of flight trajectories of pigeons can reveal that topographic features influence their flight paths. Recording electrical brain activity that reflects attentional processing could indicate objects of interest on route yet not causing changes in the flight path. Therefore, we investigated in homing pigeons whether crossing particular visual landmarks when homing from a familiar release site is associated with changes in EEG.

Results: Birds carried both data-loggers for recording GPS position and EEG during flight. First, we classified characteristic EEG frequencies of caged birds and found five main bands: *A*: 0-3, *B*: 3-12, *C*: 12-60, *D*: 60-130, and *E*: 130-200 Hz. We analyzed changes in these activity bands when pigeons were released over sea (a featureless environment) and over land. Passing over the coastline and other prominent landmarks produced a pattern of EEG alterations consisting of two phases: activation of EEG in the high-frequency bands (*D* and/or *E)*, followed by activation of *C*. Overlaying the EEG activity with GPS tracks allowed us to identify topographical features of interest for the pigeons that were not recognizable by distinct changes of their flight path.

Conclusions: We provide evidence that EEG analysis can identify landmarks and objects of interest during homing. Middle-frequency activity (*C*) reflects visual perception of prominent landmarks or objects of interest, whereas activation of higher frequencies (*D*, *E*) is linked with information processing at a higher level. Activation of *E* bands is likely to reflect an initial process of orientation, not necessarily linked with processing of visual information.

Introduction

The ability of homing pigeons to return from unfamiliar locations is a phenomenon for which underlying mechanisms are only partially known and still much debated. Pigeons have been shown to use the position of the sun [1-3], the Earth's magnetic field [4, 5] and olfaction [6, 7] to home from unfamiliar places (for review see [8]). Over familiar landscapes, they rely also on visual cues [7, 9, 10], such as highways and crossroads [11, 12]. Our previous study has shown that the ability to use such visual cues is a learned strategy [11].

The obvious flexibility of the pigeons in using different cues for successful homing poses a problem for interpreting homing data, even when data are recorded using GPS tracking. While this technique permits a very detailed reconstruction of the bird's flight and has allowed detection of patterns in routes such as alignment to topographical features [9, 11], the approach is limited in terms of explaining the cognitive processes behind such patterns. For example, when a pigeon does not show a change in flight path while crossing potentially interesting cues, it is impossible to recognize whether it does not perceive them or whether it chooses to ignore them by relying on other navigational cues**.**

A possible approach to evaluate the impact of navigational relevant cues is to analyze brain activity during flight and merge it with the GPS tracks. The goal of the present study was to provide evidence that electrical changes in the pigeon's brain during its homeward journey can signal the visual perception of landmarks that are relevant for navigation. We expected that EEG recording could reveal reactive changes in brain activity caused by stimulus perception and subsequent processing of the information.

Attentional processing and other key functions of the brain can be analyzed by recording changes in the EEG within different frequency bands [13, 14]. To record the electrical brain activity in freely flying pigeons, we constructed "neurologgers" [15, 16], small electronic devices which are able to store EEG and neuronal activity in pigeons flying up to 100 km, recording their flight paths concurrently by miniaturized GPS loggers with a spatial accuracy of ±5 m [17, 18] and temporal resolution of 1 sec (see Supplemental Experimental Procedures, and Figure S1), while the EEG was recorded from epidural electrodes.

First, we studied and characterized the EEG of pigeons in an outdoor cage while they were observing the environment. In order to recognize the EEG signatures of visual inputs, recordings were done either with both eyes open, or one eye (left or right) occluded. Since the visual pathways of the pigeon convey information from one eye preferentially to the opposite hemisphere, this procedure also served to determine the degree of asymmetrical hemispheric processing of visual stimuli [19].

In a second step, we performed a series of releases from the sea during which the pigeons initially were forced to fly over the sea (a relatively featureless visual environment), and then fly over a landscape containing familiar and navigational relevant landmarks, as evidenced by previous GPS tracking studies [11]. This served to identify different EEG signatures of information-poor and information-rich areas, and to search for EEG changes when the pigeons crossed familiar landmarks.

In a third study, the birds were released from a short distance but forced to cross two familiar landmarks. This was conducted to verify the findings from the sea releases and to analyze the electrical changes in the left and right hemisphere while crossing such a landmark. The pigeons were also released in flocks to estimate whether the diverting attention to companions and to landmarks might affect visually-dependent EEG activity.

We identified prominent frequency bands, partially differing from those observed in mammals, and we could demonstrate predictable changes in the high-frequency range during release and over relevant landmarks. This permitted identification of topographical features of interest for pigeons during their homing flight.

Results

Identification of prominent EEG frequency bands and assessment of hemispheric asymmetries

These studies served to determine prominent frequency bands in the EEG spectrum and identify those responding to visual stimulation. Overall, analysis of the pigeon EEG revealed the following frequency bands, labeled with Latin letters from A to E for future reference: ***A*: 0-3, *B*: 3-12, *C*: 12-60, *D*: 60-130,** and ***E*: 130-200 Hz** (Figure 1). We

avoided the use of Greek letters as commonly used in mammals, since the interval ranges we described do not coincide with those of mammalian studies.

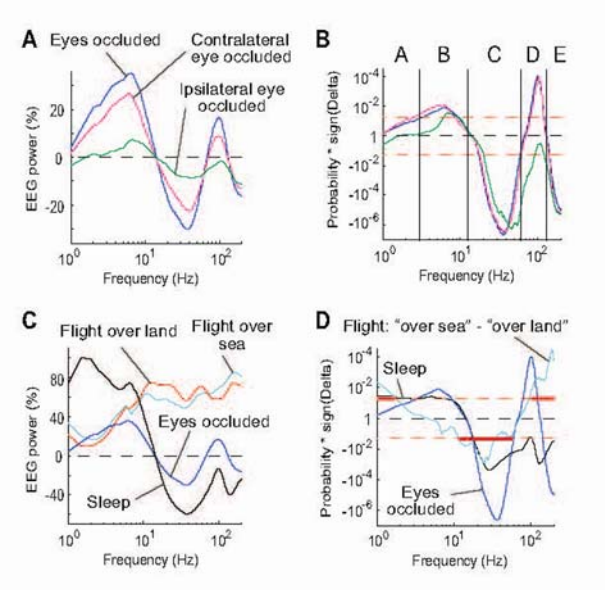

Figure 8. **High-frequency EEG is behavior-dependent in pigeons.** (A) Shifts in EEG power caused by eye occlusion. (B) Probability of difference multiplied by the sign of deviation. Red dotted lines mark $P = 0.05$. Letters *A*, *B*, *C*, *D* and *E* denote frequency ranges 0-3, 3-12, 12-60, 60-130, 130-200 Hz respectively. (C) Deviations in EEG power of four different states: eyes occluded, sleep, flight over land and sea. (D) Statistical significance of deviations in sleep and with both eyes occluded from the open eyes state; significance of difference between flights over sea and over land.

To determine frequency bands sensitive to visual stimulation, pigeons were housed alone in an outdoor cage and their visual input was reduced by occluding with eye-cups the left, the right, or both eyes. The EEG activity was recorded from four electrodes, two placed over the left and two over the right hemisphere (Figure S2). Occlusion of the contralateral eye (opposite to the recording electrodes) caused almost the same alternation of the EEG spectrum as occlusion of both eyes: a decrease of EEG power in the frequency bands 14-70 and 120-200 Hz and an increase of EEG power at other frequencies (Figure 1A). In contrast, occlusion of the ipsilateral eye (same side as the electrodes) caused much smaller variations of the spectrum (Figure 1A). The deviations were statistically significant ($P < 0.05$) in the case of both eyes occluded in bands 3-9, 18-60, 80-120 and 140-200 Hz (Figure 1B). The magnitude of changes in the left hemisphere was slightly larger than in the right (Figure S3), indicating the well-known functional asymmetry of the avian brain [20-23]. However, the EEG asymmetry in cases of symmetrical input was much smaller than the one observed after asymmetrical visual input (Figure S3). Releases of pigeons with left or right eyes occluded confirmed that asymmetrical visual input causes asymmetrical brain activation also in flight (Figure S4). Sleep in pigeons (Figures 1C and 1D) showed that the decrease of medium-to-high frequency activity (>20Hz) may reflect their sleep state better than the occurrence of slow waves (the main mammalian indicator of sleep).

We also studied in selected pigeons the impact of neck muscle activity on EEG. Muscle artifacts were observed chiefly in the low-frequency bands *A* and *B*, but were absent or negligible in the middle- and high-frequency bands (see Supplemental Experimental Data and Figure S5).

Flight over sea and land

The aim of these studies is to reveal the influence of presence or absence of local ground objects on EEG of flying pigeons. All pigeons had been trained previously to return from the sea carrying plastic dummies or GPS loggers. Birds equipped with EEG and GPS dataloggers were then released over the Mediterranean Sea - providing a visually featureless environment - at a distance of 18 km from the coast, about 30 km from their loft (Figure 2). Three releases were performed in one-week intervals. The visibility between these releases was different, ranging from poor to excellent. The average duration of such flights was 37.8 ± 11.7 min (mean ± s.d.), and flight speed varied between 60-80 km/h, indicating that the additional weight of the EEG datalogger carried by the pigeons was no handicap. Further details can be found in the Supplemental Experimental Data, Figures S6 and S7.

In order to visualize the dynamics of EEG changes, GPS recorded flight segments of 1 s duration (approximately 16-22 m depending on speed) were color-coded in sequential dots showing the deviation from the average power of a given frequency band. In comparison to the resting state, the EEG power during flight (both over sea and land) increased relative to the resting state (+60-80%) in the frequency ranges of 10-200 Hz (Figure 1C). However, over sea the power of the middle frequencies (12-60 Hz) was diminished, while both very low (0-2 Hz) and high (100-200 Hz) frequencies were increased (Figures 1C and 1D). These intervals approximately coincide with intervals previously labeled *C*, *A*, and *E*, respectively. In the frequency bands *C* and *E*, variations of power were not correlated.

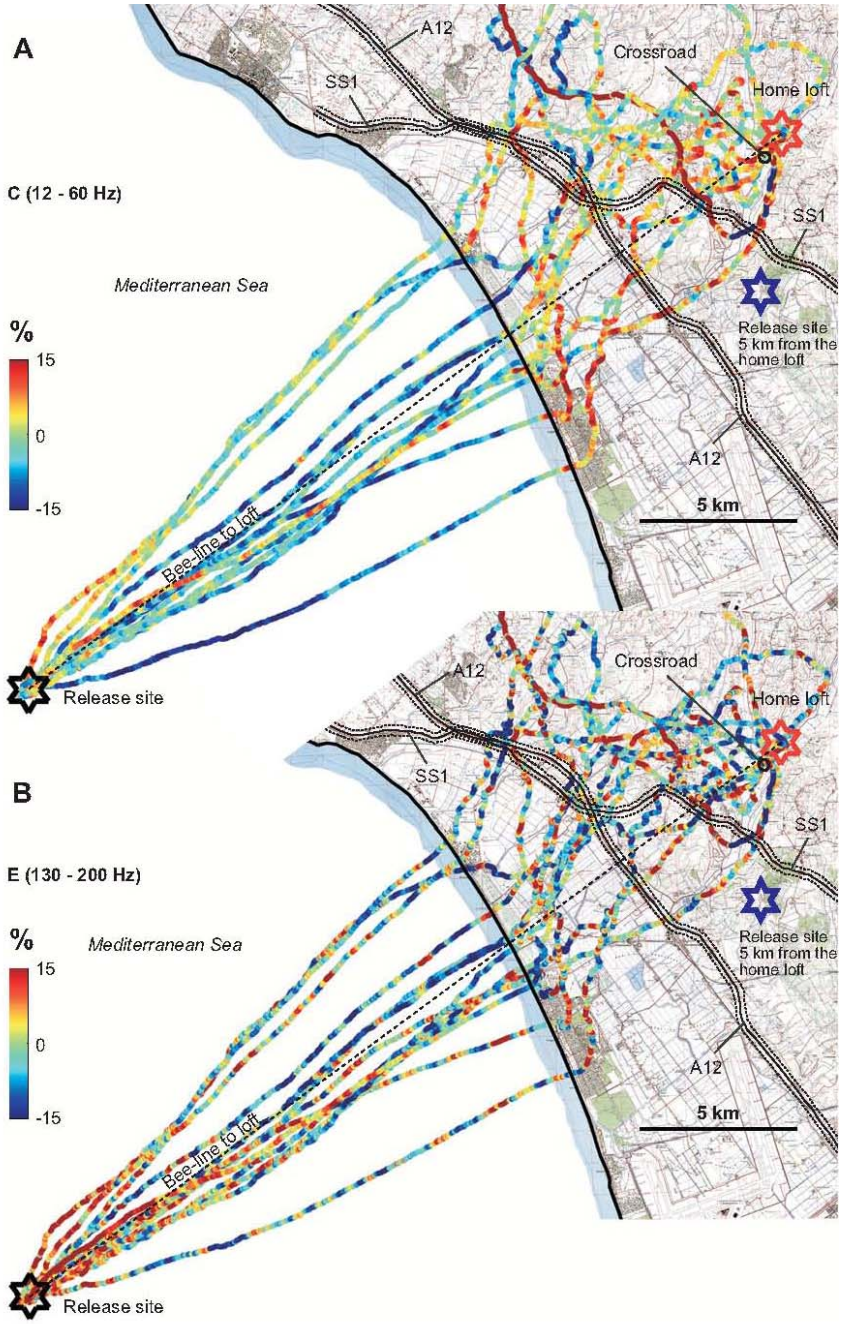

Figure 9. EEG in pigeons differs in flight over sea and over land. (A) Color-coded representation of deviation of *C* band (12-60 Hz) EEG power from the average during the flight. (B) Similar to (A) for *E* band (130-200 Hz). EEG power in the 12-60 Hz frequency range was much higher over land than over sea. In the frequency range 130-200 Hz the ratio was the opposite (EEG power over sea was higher than over land). Both differences were statistically significant, with the maximum statistical difference $P = 1.3 \cdot 10^{-3}$ reached at $f = 25$ Hz and $P = 3.45 \cdot 10^{-5}$ reached at $f = 185$ Hz for the first and the second frequency bands respectively (Figure 1D, non-paired two-tailed *t*-test, $N = 13$). It should be noted that EEG power in both frequency ranges sometimes increased when animals crossed longitudinal features like the coastal line and highways A12 and SS1 along the coast. These highways have been observed to be used by pigeons as guidance from NW to the loft [11]. Dotted lines along the highways mark 150-m areas near them.

Variations in EEG power when flying over sea

The color coded trajectories in Figure 2 show that the high EEG power in *C* and *E* frequencies near the release site decreased with time after release. Quantitative analysis of EEG in the frequency bands *C*, *D* and *E* revealed a linear decrease with distance from the release site (up to 1 km before the coast) as evident in power density (Figure 3A),

deviation from the baseline (Figure 3B), and power (Figure 3C). Afterwards values rose again. The decline in EEG power was 0.23 ± 0.51, 0.51 ± 0.48, and 0.67 ± 0.91 %/km (mean ± s.d.) for the *C*, *D*, and *E* frequency ranges, respectively (Figure 3D). The decline in all three ranges was statistically significant ($P = 0.0109$, $P < 0.0001$, and $P < 0.001$, $N = 33$, two-tailed *t*-test). However, these values were not decreasing with a similar rate. This implies again that oscillations at these frequencies reflected different brain processes that were not necessarily linked with each other. While Figure 2 shows the results from a release with excellent visibility only, a detailed comparison of the three releases under different visibility showed some subtle but significant differences in the frequency ranges *C* and *D*, but none for frequency range *E*. The observed differences were possibly related to visibility, flight experience, and motivational status (see Supplemental Experimental Data and Figures S6-S8).

Variations in EEG power when flying over land: increase in middle frequencies (C range) occur over landmarks

Figures 3A-3C show a clear peak of activity in the *C* band when pigeons crossed the coastline. To check whether such activation occurred also over other main landmarks we analyzed the changes in the *C* band while the pigeons were crossing other familiar landmarks known to be followed by pigeons from earlier studies: i.e. the highways A12 and SS1 ("Aurelia") and a point passed by many pigeons just before arriving at the loft ("crossroad"). These locations are marked in Figure 2. We calculated the EEG power in an area of ± 150 meters from the landmark that approximately coincides with the width of the peak of EEG activation over the coastline in Figure 3C. The borders of these areas are labeled by dotted lines along highways and by a circle ($d = 300$ m) over the crossroad in Figure 2. Seventeen tracks (out of 33) crossed the area of the circle. To check whether

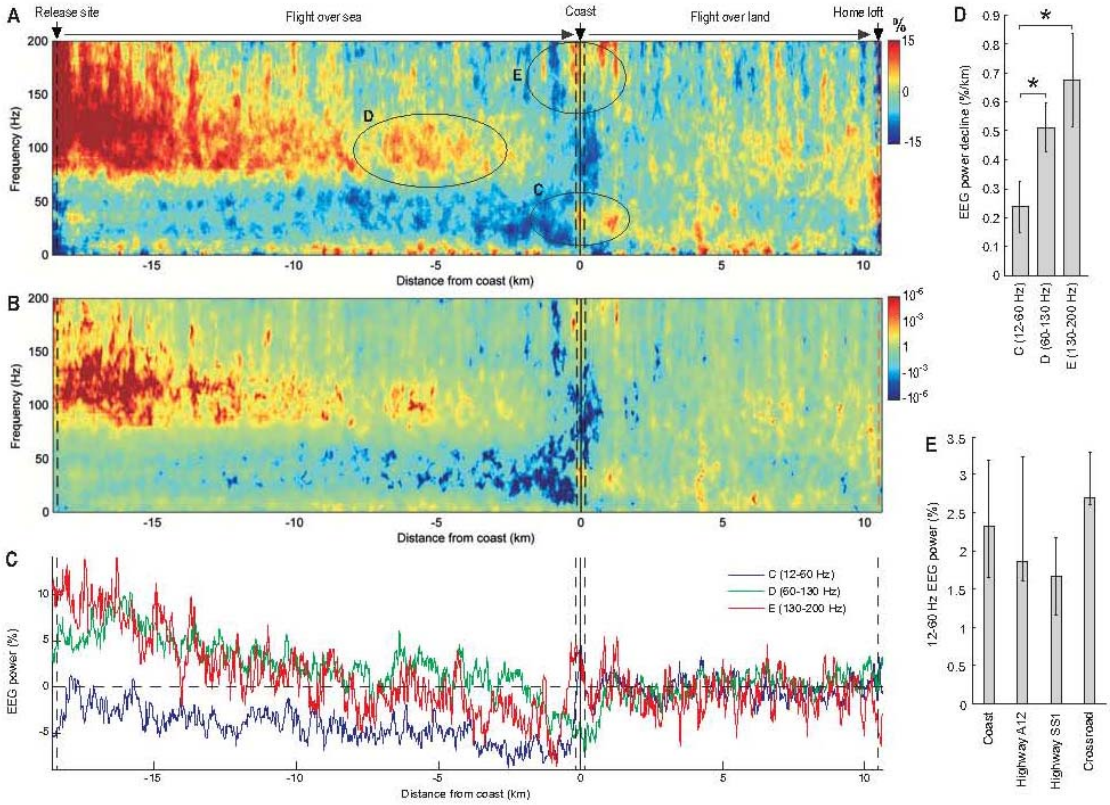

Figure 10. **Dynamics of EEG power over sea and coast.** (A) Distance-frequency (DF) representation of EEG power (in % from average EEG power over land). Ovals emphasize separate activations in three frequency bands *C* (12-60 Hz), *D* (60-130 Hz) and *E* (130-200 Hz). Dotted lines near the coast mark 150-m area near it. Horizontal scale indicates distance to the coast. (B) DF representation of probability (bootstrap, 33 tracks) multiplied by the sign of deviation. (C) Dynamics of EEG power in three frequency bands *C*, *D* and *E*. Note a very sharp peak of activity in *C* band just over the coastal line. Activation of *E* band foreruns it. (D) Decline of EEG power over sea takes place in all frequency bands *C*, *D* and *E* (mean ± s.e.m.). However, the rate of decline is different for different frequencies. Asterisk, $P = 0.005$ (paired two-tailed *t*-test, $N = 33$). (E) Activation of C band takes place at all main landmarks on pigeon way home: coastal line, highway A12, highway SS1 and crossroad just near the home loft (median). All landmarks are plotted at the map in Figure 2. Error bars show quartiles for the medians.

changes in the EEG over such locations occurred by chance, we used a bootstrap simulation method that shifted randomly all short EEG epochs of 1 s along the trajectory over land, and calculated the probability that an observed local change in EEG power would coincide by chance with the selected locations. For details of the bootstrap simulation, see Supplemental Experimental Procedures.

The highway SS1 was crossed by 32 tracks, as one pigeon stopped before and the battery of its GPS logger was discharged. All 33 tracks with color-coded EEG power of the *C* band are plotted together in Figure S9. Clearly, in many tracks EEG activity was

increased over these landmarks. A detailed quantitative analysis of changes in the *C* frequency band power is given in Figure S10. In brief, *C* band EEG activation, measured as average in a 150-m zone around the landmarks, was statistically significant when crossing any of these locations ($P < 0.01$ for the coast, $P < 0.0001$ for highway A12, $P < 0.001$ for highway SS1, and $P < 0.000001$ for the crossroad, as estimated by bootstrap simulation). These were not the only locations eliciting activation of the C band but the ones with the highest predictability.

Activation of middle frequencies (C range) is lower in flock-flying pigeons

To analyze location-dependent *C* band activation with more replicates, we released pigeons from a distance of 5 km from the home loft. The release site (Figure 2) was chosen to force the pigeons to pass two landmarks close to the home loft, namely the highway SS1 and the small crossroad near the home loft (see satellite aerial photos of the area and landmarks in Figures S11 and S12). In order to vary the degree of visual attention paid to these landmarks, the birds were released either singly (48 tracks, Figure 4A), or in flocks of six (54 tracks with good EEG, 10 flock releases, Figure 4B). Because pigeons must divert their visual attention to their companions in order to stay in the flock, we expected them to pay less attention to landmarks.

Single birds flew significantly slower than flocks (8.3 ± 2.4 versus 5.6 ± 0.4 minutes (mean ± s.d.; $P < 0.00001$, $N_1 = 48$, $N_2 = 10$, Mann-Whitney). EEG power increased approximately 150 m before the ground features of interest (highway or crossroad) and decreased 150 m after it (Figures 4C and 4D). This was true for both single- and group-released birds over the SS Aurelia (Figure 4E, for single releases $P < 0.0000001$; for flocks $P < 0.001$), and the crossroad (Figure 4E, $P < 0.0001$ and $P < 0.0001$, respectively). The lower increase of EEG power in the flock-flying pigeons was verified by bootstrap simulation ($P = 0.045$, Figure 4E), confirming our hypothesis of visual attention diverted to companions. On the other hand, the increase of EEG power over the highway and the crossroad had significant variance (deviation from baseline in percents of baseline EEG power - over highway: 3.0 ± 3.9%, over crossroad: 4.5 ± 9.7%, mean ± sd) and did not occur in all pigeons, or varied with the day of release, particularly

near the crossroad. Consistent with the in-cage studies with eye occlusions, landmarks lying asymmetrically to the pigeon path caused stronger activation in the contralateral (opposite to the object) hemisphere. For details see Supplemental Experimental Data and Figures S13 – S16.

Can EEG analysis during flight detect objects or locations of interest for pigeons but not for experimenters?

Since the EEG activation near the crossroad immediately before the loft did not make sense in terms of navigational landmarks, we conducted a detailed analysis of left- and right-hemispheric EEG responses at this place, and at another location near the loft marked by a spot of EEG activity in a minority of birds passing there (see insets of figure 4A, Figure 5). A high-resolution satellite picture showed that there was a small valley with a farm and a cattle paddock (Figure S17). Interestingly, the increase in *C* band activity appeared more pronounced in the right hemisphere of two birds passing to the right of the farm, yet more pronounced in the left hemisphere of the three birds passing the farm at left (Figure S17). This indicates separate activation of the EEG through the eye that first perceived the farm while passing. Using bootstrap simulation, we estimated post-hoc the probability to get the measured degree of activation in the area of four squares (400 m^2, labelled "1" in Figure 5A), similarly to what we did in 150-m ranges near other landmarks. This area was crossed by six tracks. In the left hemisphere the *C* band showed significant activation (10.5 ± 4.6%, mean ± sem, $P = 0.022$), but not in the right hemisphere (3.7 ± 3.0%, $P = 0.22$. The average activation of both hemispheres was 7.65 ± 3.3%, $P = 0.021$).

Likewise, a comparison of the activity of left and right hemispheres in tracks near the crossroad (Figure 5A, 5B) showed that pigeons passing the crossroad at left had left-hemispheric activation and those flying just above it a right-hemispheric activation (area is labeled as "2" in the inset of Figure 5A). This implies that the real object of interest was not the crossroad itself but a nearby object, possibly a barn located 60 m to the left of the crossroad (see also Figure S12B).

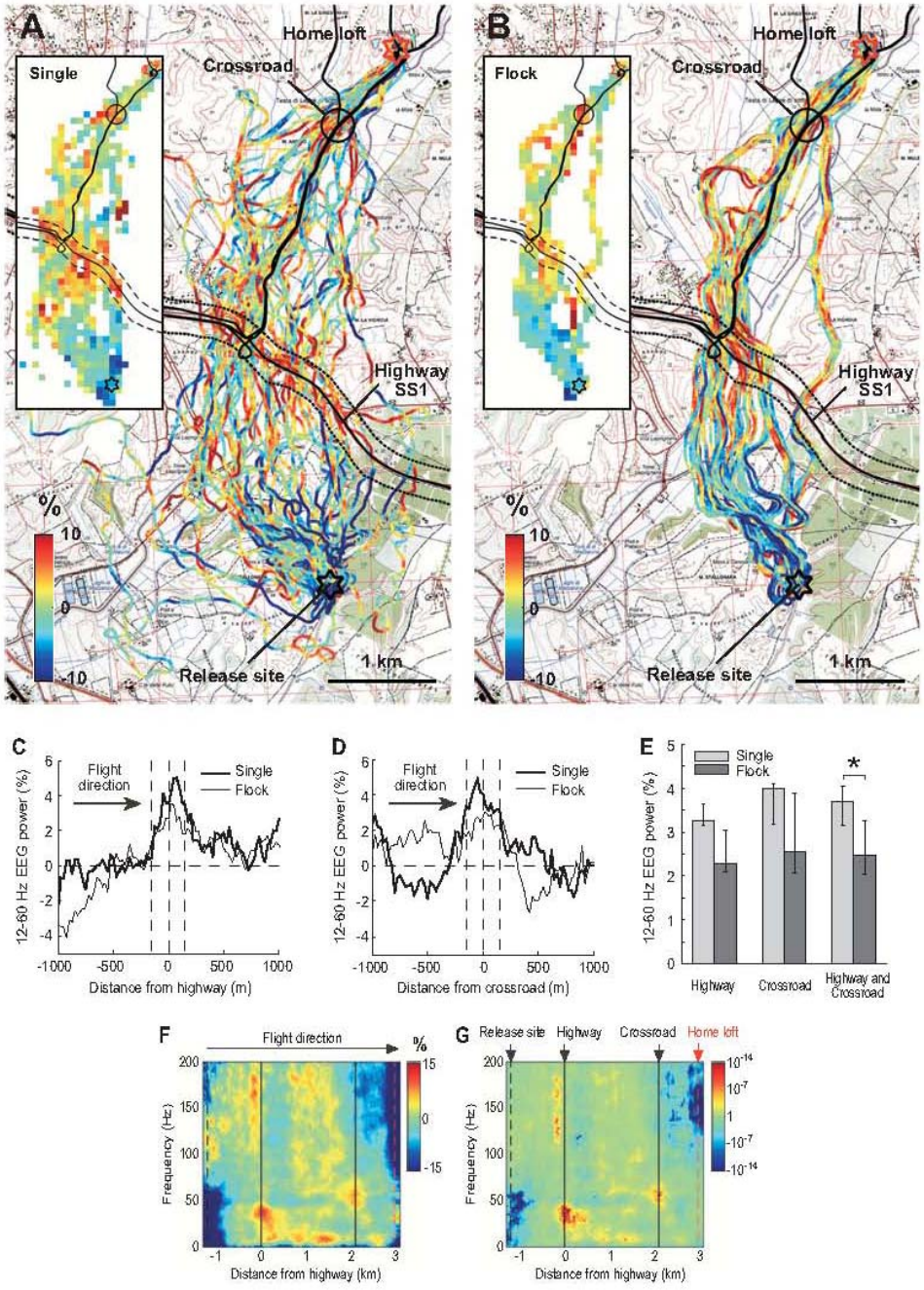

Figure 11. **12-60Hz EEG power is increased in pigeons flying singly and in flock over navigation-relevant landmarks such as highway and crossroad**. (A) *C* range (12-60 Hz) EEG power in pigeons flying along. (B) *C* range (12-60 Hz) EEG power in pigeons flying in flock. Trajectories of individual flights are plotted over topographic map. Colored segments indicated deviations from the average of EEG power in *C* (12-60Hz) range in percents from this average. Average values were calculated individually for each flight to remove influence of inter-subject and day to day variability. The circle around the crossroad and two dotted lines near the highway mark a 150-m zone near the landmarks. Insets show EEG power averaged in 100x100m squares (deviation in %). Only squares containing at least three trajectories are shown. Note the increase of EEG power - marked in orange - over the landmarks, especially in pigeons flying alone. (C and D) Dynamics of *C* range (12-60 Hz) EEG power near the highway and crossroad. (E) *C* range EEG power in 150-m zone near the landmarks. Error bars show quartiles for the medians. Asterisk, *P* = 0.05 (bootstrap, 22 birds). (F) Distance-frequency (DF) representation of EEG power (in %). Note maximum EEG power in the *E*-range 150 m before the highway. (G) DF representation of probability (bootstrap, 22 birds) multiplied by the sign of deviation.

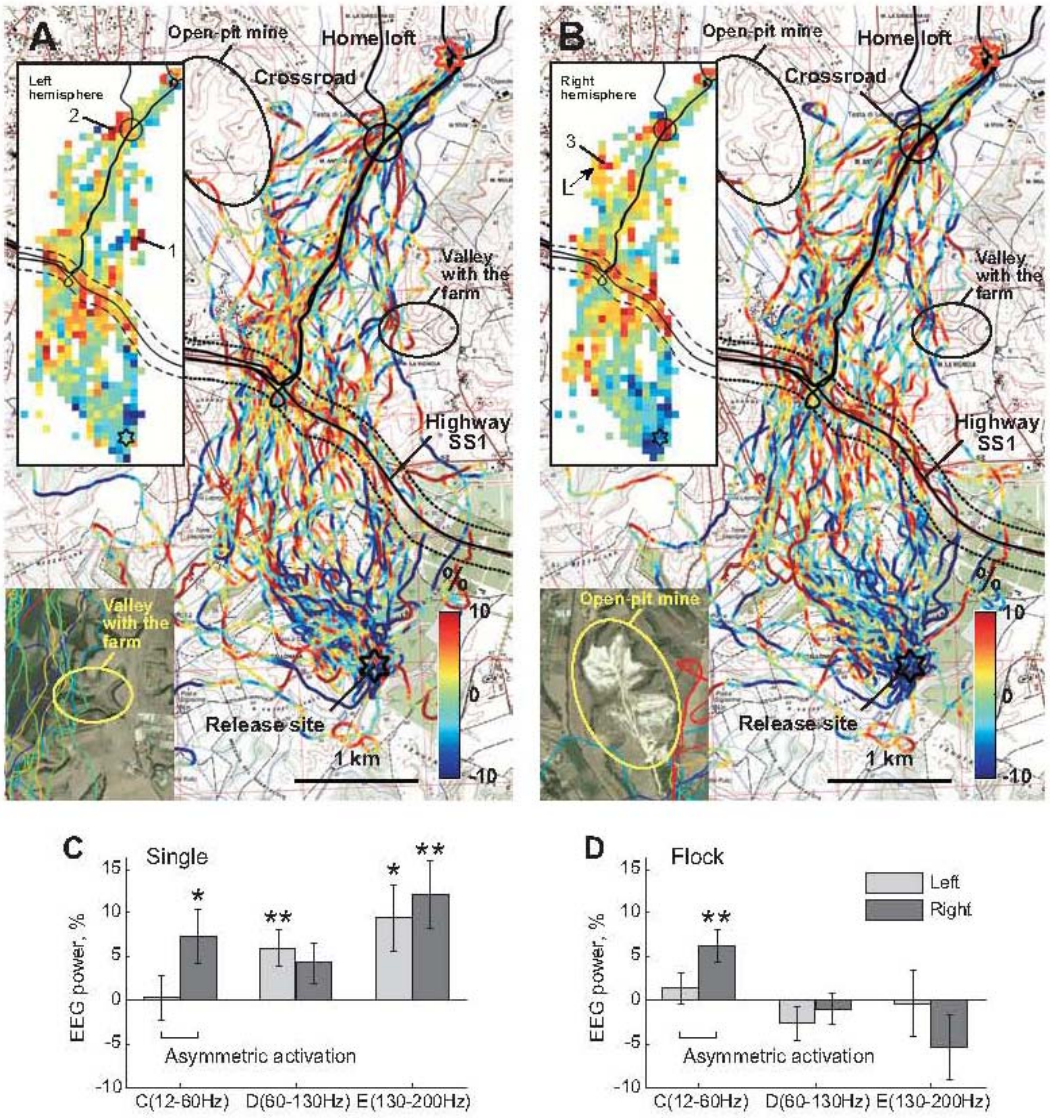

Figure 12. Changes in EEG power in the left and right hemispheres in pigeons over landmarks. (A) Map and color-coded *C* range (12-60 Hz) EEG power in the left hemisphere (in %) in pigeons flying alone. (B) The same in the right hemisphere. For explanations see legend Figure 4 where similar panels are shown. Note asymmetrical brain activation over areas labeled 1 and 2 in the inset of (A), suggesting that the place of interest was at the right side of the pigeon's track. Asymmetrical brain activation was also observed over the area labeled 3 in the inset of (B). This activation was most probably caused by well-visible open-pit mine at the left (see photo inset of (B) and Figure S11). Some increase of path entropy [24] in this area (Figure S13) can be explained by this landmark. (C) Changes in EEG power near the open-pit mine in pigeons flying alone. (D) The same in pigeons flying in flocks. Asterisks label significance of deviations from zero. One asterisk - $P < 0.05$, two asterisks – $P < 0.005$, bootstrap.

The riddle of such highly specific *C* band activation at places close to the loft and likely to be irrelevant for proper navigation was then elucidated by visiting these places: both harbored a colony of feral pigeons, that is, a place of high ethological relevance for our birds.

Can EEG changes during flight be linked to directional changes of the flight path?

In order to evaluate the usefulness of EEG changes for the analysis of spatial orientation processes, we searched for changes in flight path suggestive of re-orientation towards a new goal. One such site was a bright and well-visible open pit mine labelled as "3" in the inset of Figure 5B. The mine extended into a valley leading away from the loft. The trajectories of the flight paths (see Fig. S11) suggested that several pigeons were first attracted by the mine but re-oriented subsequently towards the loft. We first analyzed possible changes in the *C*-band to estimate the degree of visual attention paid to this landmark, and afterwards changes in the *D* and *E* bands suspected to be indicators of course-setting during the sea releases.

Six tracks passed through this area, all to the left of the open pit mine. Remarkably, the activation of *C* band in the area of three squares (300 m^2, label "3") was absent in the left hemisphere ($P = 0.91$), but present in the right ($P = 0.020$; Figure 5C). The averaged activation of both hemispheres was not significant ($4.0 \pm 2.46\%$, $P = 0.10$). Thus, passing this well-visible object seen by the left eye probably caused a predominant activation of the right hemisphere.

From the previous analysis it was known that *C* band activation can follow activation of *D* and *E* bands after a relatively small distance of about 150 m (Figure 4F, 4G and S14). This corresponds to a size of a 100 x 100 m square measured diagonally (141 m). Thus, the area where *D* and *E* band activation can be expected is a single-layer array of squares to be crossed by a pigeon just before the area of *C* band activation. In this case it consists of four squares arranged in the form of the letter "L". The letter is shown in the inset of Figure 5B, the arrow points at the array location. Ten tracks of individually released birds crossed this area. Indeed, both *D* and *E* bands were activated in this L-shaped area in either hemisphere (Figure 5C). The probabilities of deviations

were: *D* band left $P = 0.0045$, *D* band right $P = 0.060$, *D* band total: 5.52 ± 1.95%, $P = 0.0046$. Similarly for *E* band: $P = 0.012$; $P = 0.0016$; total 10.57 ± 3.39%, $P = 0.0018$.

Two flocks of six pigeons also flew over the area labeled "3" and over the adjacent L-shaped area, providing eleven tracks with complete EEG recordings. Activation of the *C* band was similar to activity in birds flying along: the left hemisphere was not activated significantly ($P = 0.39$), the right hemisphere was strongly activated ($P = 0.00052$), leading also to significant average overall activation 3.64 ± 1.49%, $P = 0.0145$ (Figure 5D). Notably, these estimates are not *post hoc*, because the areas were determined from a previous independent dataset. Contrary to birds flying alone, no activation of *D* and *E* bands was observed in flocks (Figure 5D): *D* band left: $P = 0.19$, *D* band right: $P = 0.58$, *D* band total: -1.76 ± 1.48 %, $P = 0.23$. Similarly for the *E* band: $P = 0.93$; $P = 0.15$; total -2.55 ± 3.15%, $P = 0.42$. Activation of *D* and *E* bands was also not detected on tracks before the L-shape area.

Alternations in EEG over essential landmarks were also accompanied by decrease of speed and increase of the path entropy [24], a measure of stochasticity of the trajectory (see Supplemental Experimental Data and Figure S18). However, these parameters could not indicate the location of the object of interest as clearly as it is seen from *C* band EEG activation (see Supplemental Experimental Data and Figure S19).

Discussion

This study represents the first recording and analysis of electrical brain activity temporally integrated with large-scale navigational movements in free-flying birds. The changes of activity in revealed frequency bands *C* (12-60 Hz), *D* (60-130 Hz), and *E* (130-200 Hz) in relation to release site and landmarks are summarized in Table 1. Resuming, *C* band was activated always at landmarks or places of high ethological interest, but never at release sites. Its activation could be unihemispheric. The *D* band appeared activated when a flock was formed, at sea release sites, and at all landmarks except the open-pit mine in flock-flyers. The *E* band was activated when a flock was

formed, at sea release sites, at the first prominent landmark on the pigeon's way after release, or when direction of the flight was changed at the open-pit mine.

While the *C* frequency band appears to be mostly associated with the visual perception of significant landmarks (both navigational and ethological), the *E* frequency band seems to be activated at moments when some additional cognitive processing is taking place. Such cognitive processing might be needed for initial orientation at sea release site when no local landmarks are present, and the analysis of sensory signals is required for large scale navigation ("sun compass", earth magnetic field, olfaction). Also, when the first significant familiar landmark appears on the pigeon's way, some adjustment of direction by this cue may be associated with *E* band activation. The *D* frequency band appears somewhat transitional between *E* and *C* and requires additional analysis (see also Supplemental Discussion for more details).

To summarize, this study has shown that the analysis of power changes in high-frequency bands of the pigeon EEG can clearly identify (previously known) visual navigational landmarks, but also ethologically relevant places. Therefore, we expect that changes in the EEG of flying pigeons will also be helpful in detecting and analyzing the navigational impact of local geophysical or airborne stimuli.

Experimental Procedures

Study area and facilities. Homing pigeons were kept in the facilities of the University of Zurich at Testa di Lepre, Italy, 25 km NW of Rome (12.27930° N; 41.93261° E). All birds underwent regular training in small flocks or individually from all directions within 50 km from the loft.

Subjects. 26 two- to three-year-old pigeons of both sexes were used. All procedures were approved by the Swiss (Cantonal Veterinary Office) and Italian authorities (Ministero della Salute and Istituto Superiore di Sanità, D.L. vo 116 del 27/01/92) for animal welfare.

Surgery. Gold-covered watch screws were used for epidural EEG recording.

Data acquisition. EEG and EMG were amplified, filtered, digitized by the Neurologger (http://www.vyssotski.ch/neurologger2) and stored in its memory with a rate of 400 samples per second. Starting time was recorded with the accuracy of 1 sec synchronization with the GPS data. Downloaded data from both loggers were analyzed using the MATLAB environment (MathWorks, Natick, MA, USA) with the help of custom written scripts and EEGLAB package [25].

GPS recordings. Between releases, birds wore PVC dummies of the same weight as the GPS in order to habituate them to the load. The total load did not exceed 30 g. For experiments, the dummies were replaced with GPS-loggers (Newbehavior, Zurich, Switzerland; Technosmart, Rome, Italy) just before the release, and placed again on the bird after retrieving the GPS at the loft. Pigeons were transported to the release site by car in a well-ventilated, visually-shielded transport cage. Prior to release, GPS-loggers and neurologgers were activated, and the birds were placed individually in a small releasing crate permitting scanning of the environment. They were not tossed but allowed to leave the crate after opening of the cover. This helped to estimate the flight motivation of the pigeon.

Supplemental Data

Supplemental Data include 20 figures, Supplemental Experimental Data, Supplemental Experimental Procedures, one Table, Supplemental Discussion and Supplemental References.

Acknowledgements

We thank Cesare and Maria Calderoni and Hans Cattin for expert care of pigeons and Irina Panova for helping with Neurologger development. This work was supported by the Swiss National Science Foundation, SCOPES 111081, the NCCR “Neural Plasticity and Repair”, and the Swiss Homing Pigeon Foundation.

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Publications

Included in this thesis:

Dell'Ariccia G., Dell'Omo G., Wolfer D. P. & Lipp H.-P. 2008. Flock flying improves pigeons' homing: GPS track analysis of individual flyers versus small groups. *Animal Behaviour* 76 (4): 1165-1172.

Dell'Ariccia G., Dell'Omo G. & Lipp H.-P. 2009. The influence of experience in orientation: GPS tracking of homing pigeons released over the sea after directional training. *Journal of Experimental Biology* 212: 178-183.

Dell'Ariccia G., Costantini D., Dell'Omo G. & Lipp H.-P. Waiting duration before being released increases the motivation to home in homing pigeons (*Columba livia*). *Submitted*.

Vyssotski A. L., Dell'Omo G., **Dell'Ariccia G.**, Abramchuk A. N., Serkov A. N., Latanov A. V., Loizzo A., Wolfer D. P. & Lipp H.-P. 2009. EEG responses to visual landmarks in flying pigeons. *Current Biology* in press.

Other publications:

Dell'Ariccia G., Lipp H.-P. & Dell'Omo G. 2005. Spostamenti dei piccioni romani: uno studio preliminare con il GPS. *Alula* XII (1-2): 97-102.

Costantini D., **Dell'Ariccia G.** & Lipp H.-P. 2008. Long flights and age affects oxidative status of homing pigeons (*Columba livia*). *Journal of Experimental Biology* 211: 377-381.

Dell'Ariccia G., Dell'Omo G., Massa B. & Bonadonna F. First GPS-racking of Cory's shearwaters in the Mediterranean Sea. *Under revision*.

Costantini D., **Dell'Ariccia G.** & Lipp H.-P. Serum oxidative stress markers correlate with flight duration in homing pigeons (Columba livia) flying a same distance. *Submitted*.

Dell'Ariccia, G., Lage, A., Wolfer, D. P. & Lipp, H.-P. How many members contribute to navigation? A high resolution-GPS study on respective positions in group flying homing pigeons. *In preparation*.

Poster Presentations and Talks

1. **Dell'Ariccia G., Lipp H.-P. & Dell'Omo G.** (2005) Movements of feral pigeons living in Rome: a preliminary study with GPS - Spostamenti dei piccioni romani: uno studio preliminare con il GPS. 1st Conference on Birds in Rome. Rome, Italy. (Poster)

2. **Dell'Ariccia G., Dell'Omo, G. & Lipp, H.-P.** (2006) GPS tracking of homing pigeons released over the sea after directional training. XXII Conference of the Italian Society for the Study of Animal Behaviour. Erice, Italy. (Poster)

3. **Vyssotski, A., Dell'Ariccia, G., Dell'Omo, G.,Wolfer, D. P.& Lipp, H.-P.** (2007) Alterations of EEG power in flying pigeons reflects reference to navigation-relevant landmarks such as crossings and roads. Society for Neuroscience. Neuroscience Meeting Planner. Program No. 839.23. San Diego, California. (Poster)

4. **Dell'Omo, G., Dell'Ariccia, G., Bonadonna, F., Vyssotski, A. & Massa, B.** (2007) GPS tracking of Cory's shearwaters in Southern Mediterranean. 31st Annual Meeting of the Waterbird Society. Barcelona, Spain. (Poser)

5. **Dell'Ariccia G.** (2009) Social effects in directional choice of flock-flying birds. GPS track analysis of individual flyers versus small groups. ESF COMPCOG Meeting, Testa di Lepre, Italy "Large-Scale Navigation in Birds and Mammals". (Talk)

6. **Dell'Ariccia, G., Lage, A., Wolfer, D. P. & Lipp, H.-P.** (2009) How many members contribute to navigation? A high resolution-GPS study on respective positions in group flying homing pigeons. 7th Conference of the European Ornithologists' Union 2009, Zurich, Switzerland. (Symposium)

Abstracts

1. Movements of feral pigeons living in Rome: a preliminary study with GPS

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Classical methods for studying the movements of feral pigeons in towns are mostly based on direct observations of ringed birds (capture/recapture methods). The distances covered during the daily movements range from few hundred meters (Barcelona) up to 30 km (Bratislava). In this study eight feral pigeons were fitted with miniature GPS data loggers, and their position recorded every ten seconds during repeated daily sessions. Similarly to the situation in Barcelona the daily movements were restricted over a narrow area confirming the limited mobility of the birds. In fact, pigeons were able to exploit different food sources in a short radius of about 150 m from a central area. There was also a wide inter-individual variability in the timing and pattern of movements, some birds moving at distances of up to 700 meters. The analysis of GPS data revealed that the time spent flying by feral pigeons was very limited compared for example to the spontaneous free flight of homing pigeons. A much bigger sample size is needed for assessing the temporal dynamics of the movements and possible sexual differences.

2. GPS tracking of homing pigeons released over the sea after directional training

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Two groups of homing pigeons were trained several times from two releasing sites situated about 30 km from the loft in a symmetrical position: one group was always released from the North and the other from the South. Initially, during the training, pigeons were released in small flocks, then after four of such releases, they were repeatedly released in single tosses. For determining the influence of the directional training on the homeward journey when released from a new location we carried out an experimental release of the birds equipped with GPS data-loggers from the sea. The reason for releasing the birds over the sea was to avoid possible influence of the landscape. The release point was in open sea, 30 km West from the loft, and about 20 km from the coast (not visible from the boat).

The GPS tracks clearly showed that pigeons trained from the South flew Northwards and reached the coast on the North of the beeline from release site to loft. The opposite figure emerged for pigeons trained from the North. Average deviation from the beeline between the releasing point and the loft were similar in magnitude but in opposite directions for the two groups of pigeons, without any exception. Moreover, pigeons were unable to correct their direction until they reached the coast, suggesting an importance of far landmarks as a navigational reference for stabilizing the compass direction.

This confirms that previous directional training deeply influences the compass direction taken by pigeons.

3. Alterations of EEG power in flying pigeons reflect reference to navigation-relevant landmarks such as crossings and roads.

Vyssotski, A.[1], Dell'Ariccia, G.[1], Dell'Omo, G.,Wolfer, D. P.[1,2] & Lipp, H.-P.[1]
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Analysis of electroencephalograms is widely used in humans and animals for evaluating the type and localization of brain activation, the effects of external sensory stimulation, or the irrelevance of specific stimuli used. It has been shown that pigeons sometimes follow large roads and use landmarks as turning points during their homeward journey (Curr Biol 14: 1239-1249, 2004). The goal of current study was to investigate possible changes in electrical brain activity associated with flying over typical ground features. Additionally, we compared EEG of birds traveling in a flock with birds flying singly. We tested the hypothesis that birds flying in a flock should pay less attention to topographical landmarks, with the exception, perhaps, of the leading bird. Finally, in order to compare the influence of topographical landmarks on EEG, we released the birds in a featurless landscape, i.e. from a boat in the middle of the Mediterranean sea, to force them to fly half their home journey over sea surface, and the other half - over land. To record EEG in the flying pigeons an ultra-light (2g) four-channel EEG/single-unit recorder ("Neurologger") has been developed (www.vyssotski.ch/neurologger2). The EEG recorded the electric brain activity from the cortex in each of the two hemispheres. The possibility of recording the EEG in a flying bird has been already shown (J Neurophysiol 95:1263-1273, 2006). In addition to the Neurologger the pigeons were also equipped with a small GPS datalogger which recorded the precise position of animal during its journey (www.newbehavior.com, for the last versions see also www.technosmart.eu). The data recorded by the Neurologger revealed that the brain areas monitored by the electrodes were activated when the birds crossed roads and road junctions. As expected, pigeons flying in flocks appeared to pay less attention to ground features than when flying singly. However, this tendency was quite weak and not statistically significant because of high variability in data. A significant difference between single and flock flights was observed only at two points: near the release site and near the home loft. At these places a typical increase of brain activity was observed only in flocks. This, perhaps, might reflect some strength in social interactions at the moment when pigeon form a flock and some excitement/competition on arrival. As

expected, when flying over the sea pigeons had reduced brain activity compared to when flying over the land.

4. GPS tracking of Cory's shearwaters in Southern Mediterranean

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The daily foraging activity of Cory's shearwaters breeding in the island of Linosa was investigated during the first month of the chick rearing period. Ligthweight (< 9 g) GPS dataloggers (www.technosmart.eu) were attached on the back of the birds and recorded the flight trajectory at intervals of 1 fix/20 sec. In addition, two GPS were also used for obtaining high-resolution reconstruction of the flying and food searching activity at 4 fixes/sec. Birds concentrated at sea, 500-1500 m in front of the main breeding area before sunset then, they reached their burrows during the night, and again after leaving the nest and before moving for the foraging trip.

During the study period the birds moved mostly towards South-East but two birds, however also moved towards North-East. None of the birds tracked moved Westwards. Wind direction was mostly from North-West during the whole study period. High resolution tracking allowed to detect details of the flight dynamics and fishing activity.

5. Social effects in directional choice of flock-flying birds. GPS track analysis of individual flyers versus small groups

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The effects of aggregation in navigating animals have generated growing interest in field and theoretical studies. According to the "Many-wrongs principle" group cohesion allows a more accurate navigation because individual errors are mutually corrected through information pooling (Bergman and Donner, 1964; Simons, 2004) but until now the few studies on the effects of group flying on the performance of homing pigeons (*Columba livia*) have led to controversial conclusions (Keeton 1970; Tamm 1980), chiefly because of the lack of appropriate technology to follow pigeons during their entire homeward flight. Therefore, we used GPS data-loggers in six highly pre-trained pigeons from a familiar release site first by releasing them six times individually, then six times as a group from the same site, and finally, again six times individually (Fig. 1). Flight data showed that the homing performance of the birds flying as a flock was significantly better than that of the birds released individually. When flying in a flock, pigeons showed no resting episodes, shorter homing times, higher speed, and almost no circling around the start zone in comparison to individual flights. Moreover, flock-flying pigeons took a nearly direct, "beeline" route to the loft, whereas individually flying birds preferred to follow roads and other longitudinal landmarks leading towards the loft, even when it caused a detour. Our results show that flying in small flocks has an important positive effect on homing performance, in terms of navigational accuracy, speed, and motivation, even in releases from highly familiar release sites. GPS tracking evidences that pigeons can dynamically shift between different coexisting strategies: individually flying pigeons show a greater reliance on topographical features for homing, keeping habitual home routes, while flocks tend to adopt a compass-based navigation.

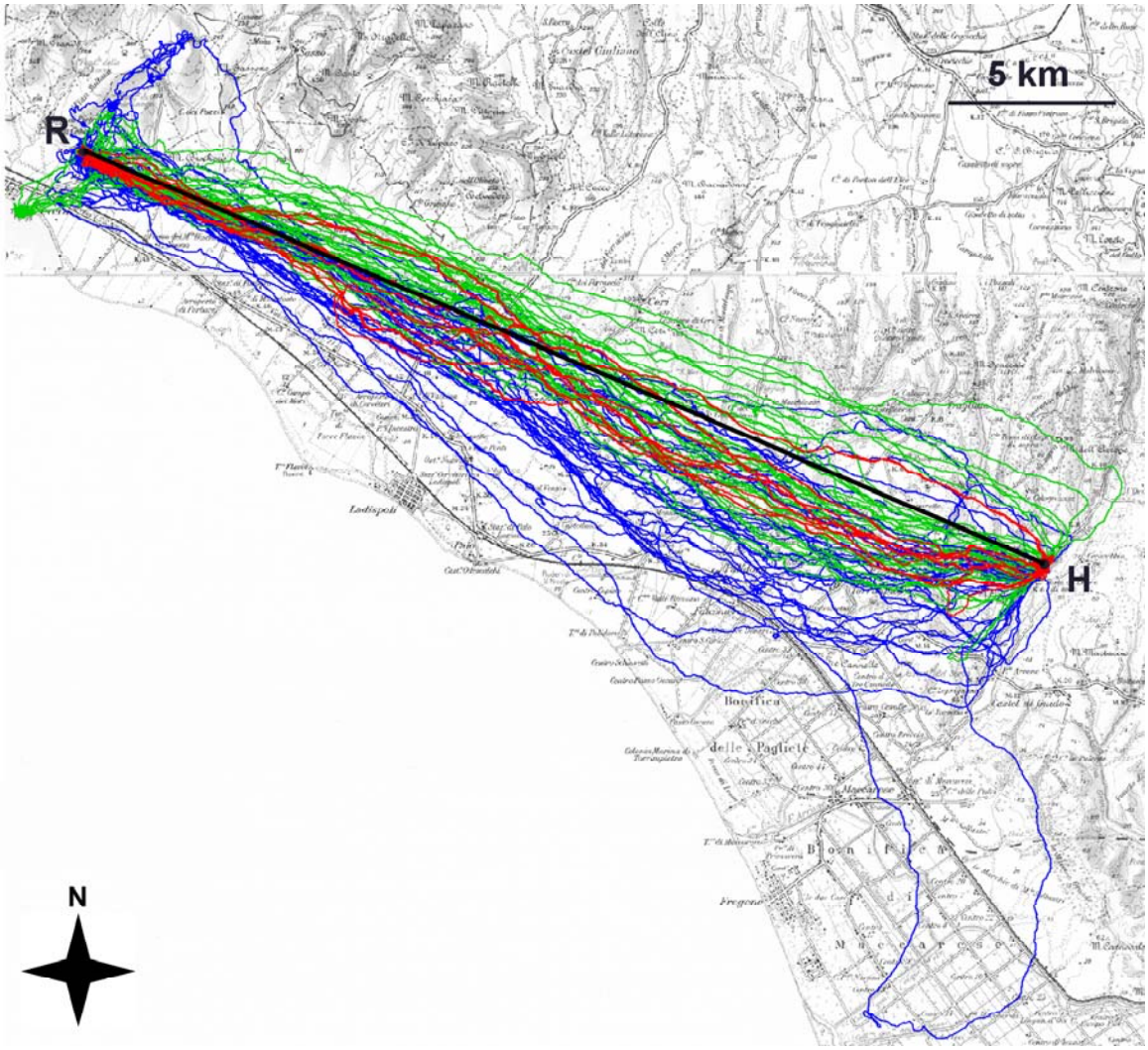

Figure 1. GPS tracks of homing pigeons between the release site (R) and the home loft (H). Blue tracks: 36 individual flights of six experienced pigeons released six times, condition S1. Red tracks: 6 group releases of the same six pigeons as a flock (apparent as one track per release because pigeons not split from the flock), condition F. Green tracks: 35 individual flights performed after the group releases, condition S2. Note the larger dispersal of flight paths under individual-release conditions S1 and S2. During S1, many flight paths coincide with roads. Tracks of group flight (red) do not coincide. During S2, some degree of coincidence of green tracks in regions devoid of roads pointing homewards is observed.

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6. How many members contribute to navigation? A high resolution-GPS study on respective positions in group flying homing pigeons.

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We used innovative GPS loggers with a very high spatial (4 meters precision) and temporal (4 fixes/second) resolution to explore the existence of a navigational leadership among homing pigeons (*Columba livia*) flying in group. Studies of performances of animals moving in group suggest two different theories on the different contribution of group members to orientation. First, the "many-wrongs principle" state that navigational errors are mutually corrected by group cohesion, and therefore all group members contribute to the final direction. The second hypothesis proposes the existence of a leader guiding all the other members. However, so far the respective contribution of group members to navigation has only been tested indirectly due to the difficulties to monitor the position of each bird during group flights. In our study, we equipped 24 groups of four to six homing pigeons with these new GPS loggers. Our results indicate that there was no fixed leader but that all group members alternated at the leading position. We observed a high variability in time spent and distance covered at the leading position among group members, unrelated to the distance or familiarity with the release site. Similarly, we found no influence of age, sex or previous experience of birds. Our results reveal that all group members contribute to orientation, supporting the "many-wrongs principle", though their respective contributions are unequal.

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